6 Principles and Practice of Analytical SFE

Steven B. Hawthorne[1] and Jerry W. King[2]

[1]*Energy and Environmental Research Center*
University of North Dakota, Grand Forks, North Dakota, USA
[2]*Food Quality Safety Research Unit, National Center for Agricultural Utilization Research, Agricultural Research Service, US Department of Agriculture, Peoria, Illinois, USA*

INTRODUCTION

The purpose of this chapter is to provide the reader with the basic understanding needed to begin implementing SFE in the analytical laboratory. The mechanics and instrumentation for SFE have been described in previous literature and/or can easily be obtained by the instrument manufacturers and will, therefore, not be emphasized. Likewise, the applications of SFE are the subject of several reviews, and interested readers should refer to recent reviews listed at the end of this chapter (Camel *et al.*, 1995; Bøwadt *et al.*, 1995; Janda *et al.*, 1993; Chester *et al.*, 1994; King *et al.*, 1992). Instead, the focus of this chapter will be to describe the interactions of various experimental parameters that are utilized in SFE in the hope that better understanding of these parameters will allow the reader to more efficiently develop and use SFE in the analytical laboratory. A special effort has been made by both authors to address common pitfalls and misunderstandings of analytical SFE that they have frequently encountered during their accumulated years of developing analytical SFE methods.

Analytical SFE can be sub-divided into two essential steps, extraction and collection. Both processes must be efficient for quantitative determinations to be possible, and it is common for new users of SFE to (for example) decide that poor overall SFE efficiencies are a result of poor extraction conditions, when the real cause of error is poor collection efficiencies. As will be demonstrated, the extraction can depend on both the matrix and analyte characteristics, and the development of quantitative SFE can be greatly improved by an understanding of these differences. In addition, SFE is often criticized because there appear to be multiple variables which must be optimized before quantitative extractions can be achieved. As will be demonstrated in the following discussions, nothing could be further from the truth. With a basic understanding of the SFE experiment and the characteristics of the sample matrix and its interaction with analytes, SFE methods can often be developed and validated in a few hours or days.

The examples and content of this chapter focus on the application areas that the authors are most familiar with: the supercritical fluid extraction of environmental matrices and foods and related agricultural products. This emphasis provides the reader with an extreme diversity in the sample matrices to which SFE can be successfully applied. Environmental samples are frequently characterized by strong analyte-matrix interactions which can be successfully overcome by optimization of extraction conditions, a change in extraction fluid identity, and the use of cosolvents. In contrast, food matrices present a somewhat different challenge to SFE, namely the presence of high water and coextractive content

Source: Practical Supercritical Fluid Chromatography and Extraction edited by Marcel Caude and Didier Thiebaut (Harwood Academic Publishers, 1999) ISBN 9057024098 Chapter 6, p. 219-282. 1999

and a strong dependence on matrix preparation prior to extraction. Whereas, the cited examples do not cover all of the application possibilities of SFE, we believe they provide adequate scenarios for illustrating the principals and practice of SFE.

I. COLLECTION OF EXTRACTED ANALYTES

The development of appropriate collection conditions for target analytes after SFE is often ignored by new users, despite the obvious fact that quantitative *extraction* conditions cannot be developed and evaluated unless the *collection* step is efficient. Thus, the first job of the analyst is to optimize the collection system and determine its efficiency for the target analytes. For bulk extraction of non-volatile analytes (e.g., determining fat content), collection of the SFE effluent can be efficiently performed in an empty vial. However, when the concentration of the target analytes is low, even non-volatile organics can be lost through aerosol formation. For volatile and semivolatile analytes (e.g., "GC-able" organics), losses from vaporization can easily occur with an improper trapping system. In the literature, many authors of SFE papers have failed to determine the collection efficiencies of their SFE system, and have reported poor extraction efficiencies when the real fault is poor collection efficiencies.

Determination of collection efficiencies requires selection of initial SFE conditions (discussed later in the text), and the generation of an appropriate spiked sample. It is especially important to note that collection efficiencies should be re-verified any time there is a significant change in the SFE conditions such as adding a modifier, increasing the fluid flow rate, or raising the extraction or restrictor heater temperature. To determine collection efficiencies, the target analytes should be spiked onto a relatively inert matrix (e.g., sand) and immediately extracted. Care should be taken that the more volatile analytes are not lost between the spiking or extraction step. For example, volatile organics (e.g., alkanes in the butane to decane range) will evaporate rapidly from sand, therefore requiring that a suitable sorbent (e.g., XAD-2 resin) be used for the spiking matrix (Yang *et al.*, 1995). If less than quantitative recoveries are achieved by this spike recovery study for volatile organics, losses between spiking and SFE should be investigated. If the recoveries are low for more non-volatile species, the SFE conditions may not be sufficient for extraction. This can be tested by extracting the SFE residue using an organic solvent to determine whether the spiked analyte was not extracted during SFE, or was not collected efficiently after SFE.

It should be noted that the problems associated with trapping are emphasized below so that the new practitioner is aware that trapping difficulties may exist, and must be considered while developing the SFE method. However, it is important to also note that both solid phase and solvent collection can be efficiently (and simply) performed for all but the most volatile analytes as long as the analyst has a basic understanding of the collection systems.

I.A. Solid Phase Collection

Solid phase trapping is normally performed by depressurizing the CO_2 and the analytes prior to the trap, and collecting the analytes from the gas (or aerosol) phase directly onto

Table 1 Collection efficiencies of optimized sorbent and solvent traps for gasoline components.

Analyte	*Percent recovery (% RSD)*[a]		
	Sorbent trap	*Solvent trap*	
	5°C	*Normal trapping*	*Pressurized trapping*
n-hexane	90 (4)	18 (13)	59 (14)
benzene	93 (4)	90 (6)	102 (9)
n-heptane	100 (3)	69 (10)	92 (9)
toluene	101 (3)	103 (5)	108 (6)
n-octane	101 (3)	95 (6)	102 (8)
o-xylene	101 (3)	104 (7)	106 (5)
n-decane	99 (3)	102 (7)	104 (6)
n-dodecane	99 (3)	102 (7)	102 (4)

[a]%RSDs are based on quadruplicate extractions of the gasoline components from XAD-2 sorbent resin extracted for 30 minutes with pure CO_2 at 345 bar and 80°C and a flow rate of ~1.5 ml/minute. Sorbent trapping was performed with a Hewlett-Packard 7680T SFE and 60/80 mesh Porapak Q sorbent set at 5°C. Solvent trapping was performed with an ISCO SFX-210 with 15 ml of methylene chloride (normal trapping) or with an ISCO SFX-3560 with 7 ml of methylene chloride (pressurized trapping). Results are adapted from reference 6.

sorbents such as silica gel, Florisil, or bonded phase packings; or onto inert surfaces such as glass or stainless steel beads (Ashraf-Khorassani *et al.*, 1992; Mulcahey *et al.*, 1991, 1992; Bøwadt *et al.*, 1993, 1994; Miller Schantz *et al.*, 1986; Howard *et al.*, 1993). After SFE, the trap is eluted with liquid solvents for subsequent analysis. Both cryogenic and adsorption mechanisms are active in solid phase trapping, however, cryogenic trapping on inert materials (glass or stainless steel beads) is largely unsuccessful for analytes with even a small vapor pressure. For example, Mulcahey reported only ~25% collection efficiency of decanoic acid on 5°C beads, even though the boiling point of decanoic acid is 270°C. The use of sorbent phases allows adsorption to be used to increase the collection efficiencies, and the selectivity of the adsorption mechanism can be used to gain a degree of compound-class fractionation during the SFE collection step. In addition, the choice of rinsing solvent(s) can be used to selectively elute different compound classes from the trapping system. This degree of selectivity based on the elution of the trap is a particular advantage of sorbent collection over solvent collection systems. However, solid phase trapping can be more complicated to optimize than solvent collection since the analyst must select the trapping material, the trap temperature, and the rinsing solvent(s).

Since the solid phase trapping temperature is normally controlled, the temperatures used for SFE and for the flow restrictor have less potential effect on collection efficiencies than for solvent collection systems (discussed below). With proper conditions, very volatile compounds can be collected as shown in Table 1. However, solid phase trapping is much more susceptible to analyte losses when modifiers are used during the SFE step, simply because the modifier can condense on the trap and elute the target analytes from the trap during the SFE step, rather than waiting for the normal elution step. The normal solution to this problem is to raise the trap temperature above the boiling point of the modifier (Mulcahey *et al.*, 1992; Bøwadt *et al.*, 1994), however, this procedure will also reduce the collection of volatile components. Correct selection of the trapping material can also

be used to avoid losses from modifiers on the trap. For example, Bøwadt reported poor collection efficiencies for PCBs if 5% methanol modifier was used with a silica trap (65°C), but good collection efficiencies under identical SFE conditions using either Florisil or ODS trapping materials (Bøwadt *et al.*, 1994).

Sorbent trapping of volatile species after SFE is particularly sensitive to the nature of the extraction fluid and trap temperature. Taylor *et al.* (1994) have shown, by means of gas chromatographic measurements (i.e., specific retention volumes), that carbon dioxide substantially reduces the breakthrough volume of many analytes on resinous sorbent media relative to more "ideal" fluids. Therefore, the analyst should evaluate the possibility of analyte breakthrough from the solid phase trapping media along with the extraction fluid of choice. Avoidance of analyte breakthrough from the sorbent trap can also be minimized by lowering the trap temperature, a feature available on many commercial instruments. Figure 1 shows this principle in action for the off-line SFE/GC of analytes from diesel exhaust particulates collected on filters (Levy *et al.*, 1993). When the collection temperature of the sorbent trap is held at 5°C, the collection efficiencies for even relative non-volatile species such as C_{16} hydrocarbons is poor. However, by cooling the trap to –45°C, over 90% recoveries could be obtained.

Today off-line SFE can be conveniently integrated with commercially-available solid phase extraction (SPE) cartridges (Maxwell *et al.*, 1995). The marriage of SFE with SPE allows the analyst to further purify, or cleanup, the resultant SFE extract. In the case of food or biological matrices, this coupling is critical, due to the presence of unwanted coextractives from the SFE. Insertion of preferred sorbent in an SPE cartridge provides not only the capacity to trap the analytes of interests, but also provides for the removal of unwanted material and segregation of the desired analyte. This allows the analyst to use many of the well developed SPE schemes along with SFE to minimize solvent usage in the analytical laboratory (VanHorne *et al.*, 1985).

I.B. Solvent Collection

Solvent collection is best performed by simply inserting the outlet restrictor from the SFE instrument directly into a small volume of collection solvent (typically 3 to 15 ml). Solvent collection is mechanically simple to perform and has the advantage that a new collection system can be used for each extraction, thus reducing the chances for carryover. However, the success of solvent collection is highly dependent on the SFE conditions including the polarity and volatility of the target analyte, the solvent characteristics (including polarity, viscosity, and boiling point), the SFE flow rate, and the temperature of the restrictor. In addition, commercially-available solvent collection systems have widely-varying collection efficiencies, especially for more volatile analytes. Therefore, there is much disagreement in the literature on the abilities of solvent collection.

If properly performed, solvent collection can be quantitatively efficient for virtually all semi-volatile organics (Langenfeld *et al.*, 1992), and even for fairly volatile organics (Yang *et al.*, 1995). Solvent selection is particularly important to obtain good collection efficiencies. For example, Langenfeld *et al.* reported that methylene chloride, chloroform, and acetone are efficient collection solvents for organic pollutants ranging from polars (e.g., substituted phenols and anilines) to non-polar (e.g., PAHs). In contrast, methanol

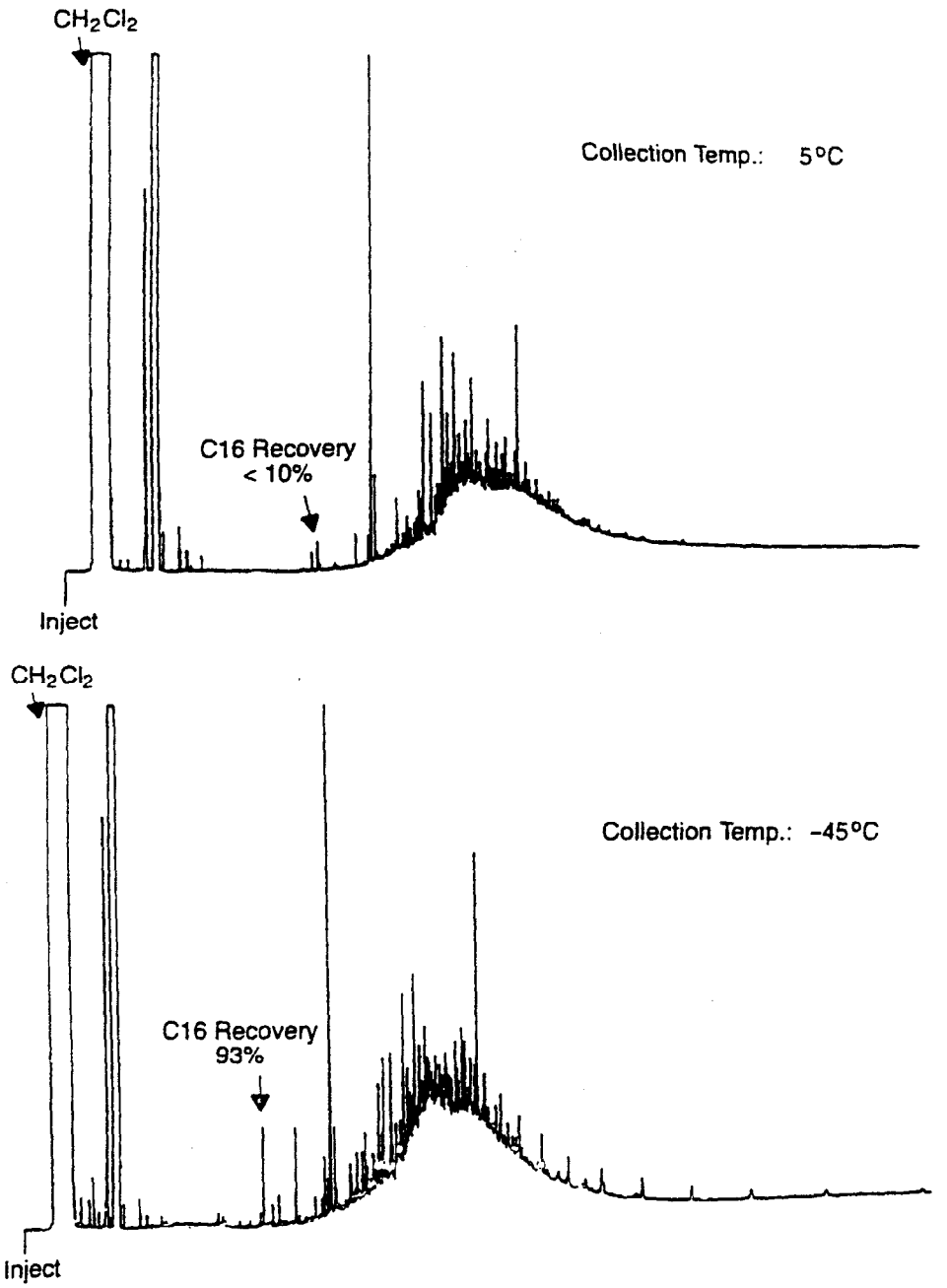

Figure 1 Off-line SFE/GC of diesel exhaust particulate from collection filters. Effect of SFE trapping temperature on collection of hydrocarbon analytes after desorption.

and hexane frequently gave poor collection efficiencies under identical SFE conditions (Langenfeld *et al.*, 1992). In addition to collection efficiencies, it is helpful to select a solvent that is compatible with the subsequent analysis. For example, methylene chloride is an efficient collection solvent for PCBs, but acetone is preferred when GC with electron capture detection (ECD) is used for the PCB determinations.

In contrast to the high collection efficiencies reported by Langenfeld, much lower collection efficiencies have been reported for the same or similar analytes using different systems for liquid solvent collection (Reindl *et al.*, 1994; Vannoort *et al.*, 1990; Meyer *et al.*, 1993; Lopez-Avila *et al.*, 1990). It should be noted, however, that properly-performed solvent collection can efficiently trap even quite volatile compounds. As demonstrated in Table 1, even species as volatile as *n*-heptane and benzene can be efficiently trapped.

In addition to solvent choice and volume, parameters which affect liquid solvent collection include the mechanical device used (direct depressurization into the collection solvent is generally more successful than depressurization to the gas phase before contacting the collection solvent) (Burford *et al.*, 1992), CO_2 flow rate (a flow of ~1 ml/min corresponds to 500 ml/min of gaseous CO_2; therefore, higher flow rates cause more losses of volatiles), height and volume of collection solvent (Yang *et al.*, 1995; Burford *et al.*, 1992), and restrictor temperature (hotter restrictors can cause loss of volatiles). While liquid solvent collections can be very efficient, the collection efficiencies experienced by investigators using different commercial and home-built systems accentuates the need for verifying collection efficiencies whenever significant changes to the extraction conditions or collection systems are made (as discussed below). Of course, this same caution should be used for any SFE collection system.

I.C. On-Line Collection

The majority of quantitative applications of SFE have involved off-line collection using either solvents or solid phase traps. However, on-line collection (where the SFE extract is directly transferred to the instrument used to analyze the extract) has been accomplished both for chromatography (most often with GC, followed by SFC and LC) (Janda *et al.*, 1993; Chester *et al.*, 1994; Maeda *et al.*, 1992; Burford *et al.*, 1994; Hawthorne, 1992; Levy *et al.*, 1992) and spectroscopy (e.g., with FTIR) (Heglund *et al.*, 1994). Since on-line techniques are the subject of a separate chapter in this book, only brief comments will be made here.

A major reason for performing on-line SFE is to facilitate the collection of species that are too volatile for the off-line collection methods discussed above. For example, SFE-GC has been used to quantitatively collect organics as volatile as butane, acetone, and methylene chloride (Burford *et al.*, 1994). On-line techniques can also greatly increase method sensitivity by allowing all of the extracted molecules to be transferred to the chromatographic system. When "real-time" monitoring of the SFE process is desired, coupling SFE with spectroscopy (e.g., SFE/FTIR) can be used to monitor extracts on the second (or less) time scale (Heglund *et al.*, 1994). One potential advantage of on-line spectroscopic systems is the ability to measure extracted components under the SFE conditions, which eliminates the mechanical problems associated with restrictor plugging and analyte collection.

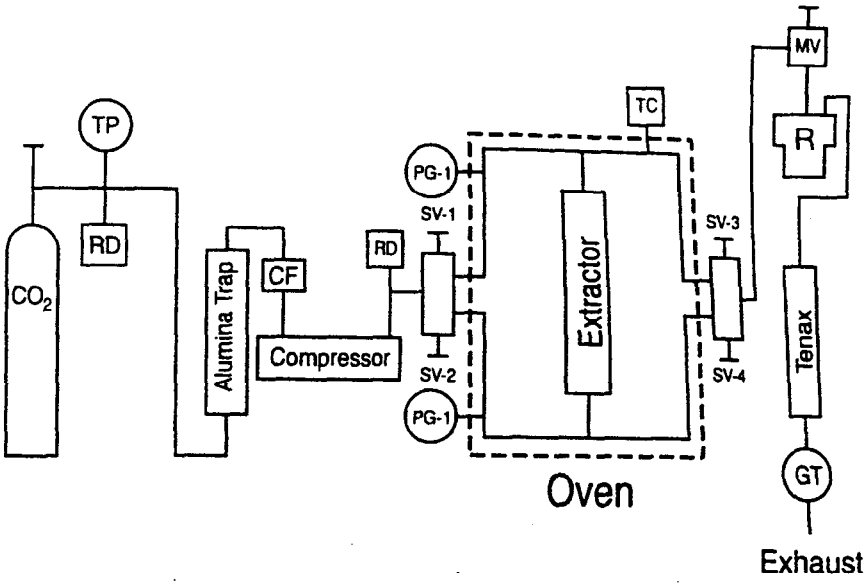

Figure 2 Schematic of a supercritical fluid extraction device for collecting non-volatiles (R) as well as volatiles on a Tenax trap.

I.D. Collection in Empty Vessels

Collection in a empty vial or vessel has been successfully practiced by a number of investigators and is particularly appropriate for bulk extraction of fat and similar exhaustive extractions. It is also applicable however, for the extraction of trace levels of analytes, such as pesticides (Hopper *et al.*, 1991), but larger collection vessels are required for capturing such trace analytes in order to minimize their loss. Avoidance of entrainment of analytes in the escaping fluid stream can be minimized by adding a glass, steel wool, or ball packing to the empty container. The chosen material should be chemically inert, provide a high surface for condensing the analyte from the rapidly expanding fluid, but allow ready desorption of the analyte after completion of the extraction. A novel scheme for inserting an open collection vessel prior to a sorbent trap for trapping of both non-volatile and volatile constituents from food matrices is shown in Figure 2. Here, the initial collector serves to capture coextracted lipid constituents, while volatile species are isolated down stream on sorbent-filled tubes (Snyder *et al.*, 1994). It is also possible to design a collection scheme that permits the concentration of volatile species in a coextracted oil under pressure.

II. SAMPLE PRE-PREPARATION

Prior to SFE, many sample matrices can be treated in an effort to ensure homogeneity, increase extraction rates, decrease flow problems such as restrictor plugging, and/or

Table 2 Extraction of aflatoxin B1 from corn.

Method	*Sample Size*	*Concentration*	*Relative Standard Deviation (N = 5)*
SFE	3.0 g	519.6 ppb	6.2
Solvent	50 g	549.2 ppb	3.5
Solvent	3.0 g	515.1 ppb	10.5

increase the selectivity of the SFE step. For many samples, no pre-preparation is required, however, there are a variety of simple steps that can be used to increase the reliability of SFE for samples having different "difficult" characteristics. Many of these pre-treatments are similar to those used for conventional solvent extractions.

II.A. Sample Size and Homogeneity

Because of the high pressures used in SFE, most (but not all) commercial instrumentation is limited to ~10 ml samples. While many samples (e.g., soils and sediments, air particulates, polymers, and leaf tissue) are homogeneous in quite small quantities (often < 1 gram) with either minimal or no preparation such as grinding or sieving, several important applications of SFE require much larger quantities of sample to ensure representative results.

The choice of sample size for any analytical determination or preparation can be more crucial than many analysts realize, and this applies equally as well to SFE. In recent years, there has been an increasing trend toward smaller sample sizes due to two factors: improved comminution methods and the desire to have smaller analytical instrumentation in the laboratory environment, i.e., smaller "footprints" on the benchtop. The latter factor, to some extent, has guided the design of the first wave of SFE instrumentation which has limited sample size on the average to around 10 grams. This limitation puts a premium on assuring sample homogeneity through mixing, grinding, and similar processes. However, such processes must not permutate the sample matrix via mechanical or thermal action so that it is not representative of the original whole sample.

An example of SFE where sample size comes into play is in the extraction of aflatoxins from corn and similar seed/grain matrices, an extraction which usually requires the use of a cosolvent to achieve suitable analyte recoveries (Selim *et al.*, 1993; Taylor *et al.*, 1993). Aflatoxins are generated on the corn matrix from infestation of the fungi, *aspergillus flavus*, and evolve and spread from a specific site, leading to a potential maldistribution of the target analyte on a single kernel of corn, throughout a corn ear, or at "hotspots" within a corn elevator. Therefore, obtaining a representative sample for SFE or any other extraction or sample preparation procedure is difficult, particularly considering that the toxicity of the analyte does not make it very amenable to many standard homogenization techniques. Table 2 shows recovery results of aflatoxin B1 from different quantities of the same corn sample for both solvent and supercritical fluid extraction. The SFE-generated result in this case was obtained on a 3-gram sample. Obviously, comparison of the SFE-extracted sample to a 50-gram solvent extracted sample would lead to a low recovery figure for the SFE process. Comparison to a 3-gram solvent-extracted sample would indicate that both extraction processes achieved similar recoveries, but is extraction of 3-gram samples

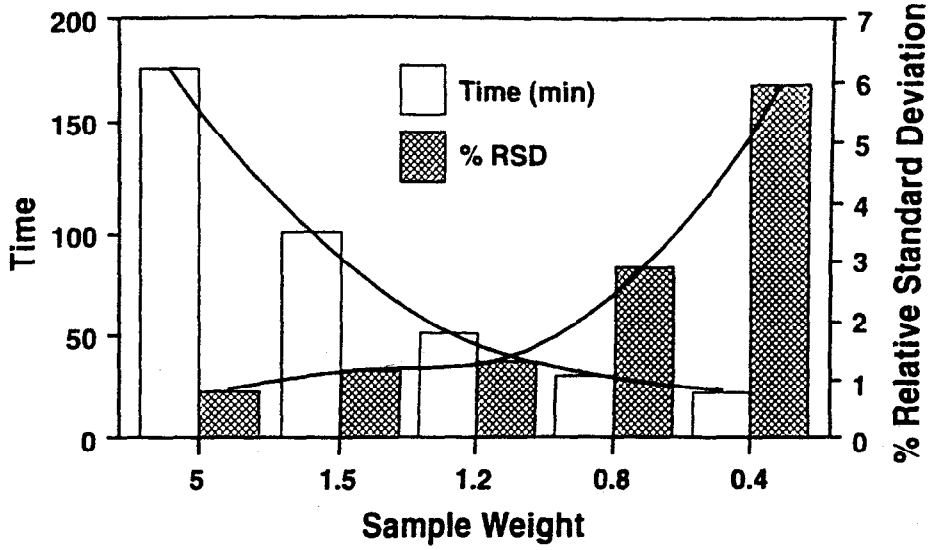

Figure 3 SFE fat extraction from potato chips. Precision vs. sample size.

the truth? Obviously, a SFE run on 50-gram sample could address this question and argues for the design of instrumentation capable of addressing the SFE of larger samples. Fortunately, some commercial equipment is available that will address this issue, including home-built equipment that is inexpensive and relatively easy to fabricate (King, 1995).

Based on statistical sampling theory (Horwitz *et al.*, 1980), using a larger sample size in any type of extraction yields more precise results. An example of this is given in Figure 3 for the determination of the fat content of potato chips using SFE. Here one sees obviously the tradeoff between sample size and precision of analysis. This has ramifications in terms of comparing SFE with older extraction methodology, which is based on much larger sample sizes. Nonetheless, with proper homogeneity, even SFE on small sample sizes has yielded very good standard deviations, comparable with those found via established methodologies.

II.B. Sample Grinding

As just discussed, an important use of sample grinding is to increase the homogeneity of samples, particularly for biological matrices. An additional use of grinding is to increase SFE rates for samples in which the target analytes are physically distributed throughout the matrix material, as opposed to being primarily on the surface of the matrix. For such samples, sample grinding increases SFE rates by reducing the distance that the analytes must diffuse through the matrix to be extracted (Bartle *et al.*, 1990). The extraction of additives from polymers is a clear case where grinding increases the rate at which analytes are removed from the matrix. However, grinding samples for which the analytes are present on the surface has no significant effect on the extraction rate. For example, we have

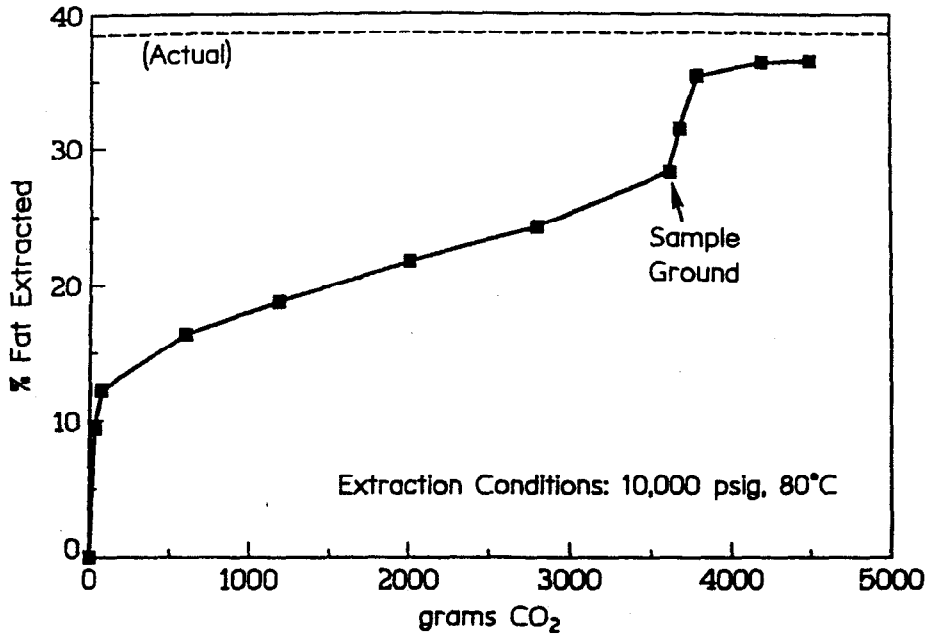

Figure 4 SFE of link sausage with SC-CO_2. Effect of regrinding sample and reextraction.

extracted PAHs and PCBs from soils and sediments both using different particle sizes produced either by grinding or by sieving, and have not observed any significant change in the extraction behavior (i.e., plots of the amounts of pollutants extracted versus time are similar regardless of soil particle size).

Inadequate sample comminution can lead to poor recoveries, particularly if a large amount of bulk analyte (fat or oil) is being recovered via analytical SFE. Figure 4 shows the result of attempting to extract all of the fat out of a large link sausage sample. In this case, fat recovery becomes rate limiting after a considerable extraction time or CO_2 passage through the meat matrix. Regrinding the sample in the extraction vessel, however, allows access to the fat enriched regions of the meat matrix and total recovery of the fat content from the link sausage matrix. One must be aware that the induction of heat through grinding may affect the enzymatic activity in a natural product type of sample and the potential loss of volatile material prior to the SFE step.

In some instances, sample grinding can be detrimental in SFE, particularly when the analytes of interest are located on the surface on the sample particle. This is particularly true when dealing with natural product samples containing many coextractives which may interfere in the analysis and require cleanup of the supercritical fluid extracted sample. In this case, SFE on the neat sample may prove more efficacious. For example, on-line SFE of the seeds of the botanical specie, *dalea spinosa*, better known as the "desert smoke tree", (a potential source of perfume ingredients), yields extracts that are dependent on the comminution of the sample (Taylor *et al.*, 1994a). As shown in Figure 5, the analytes

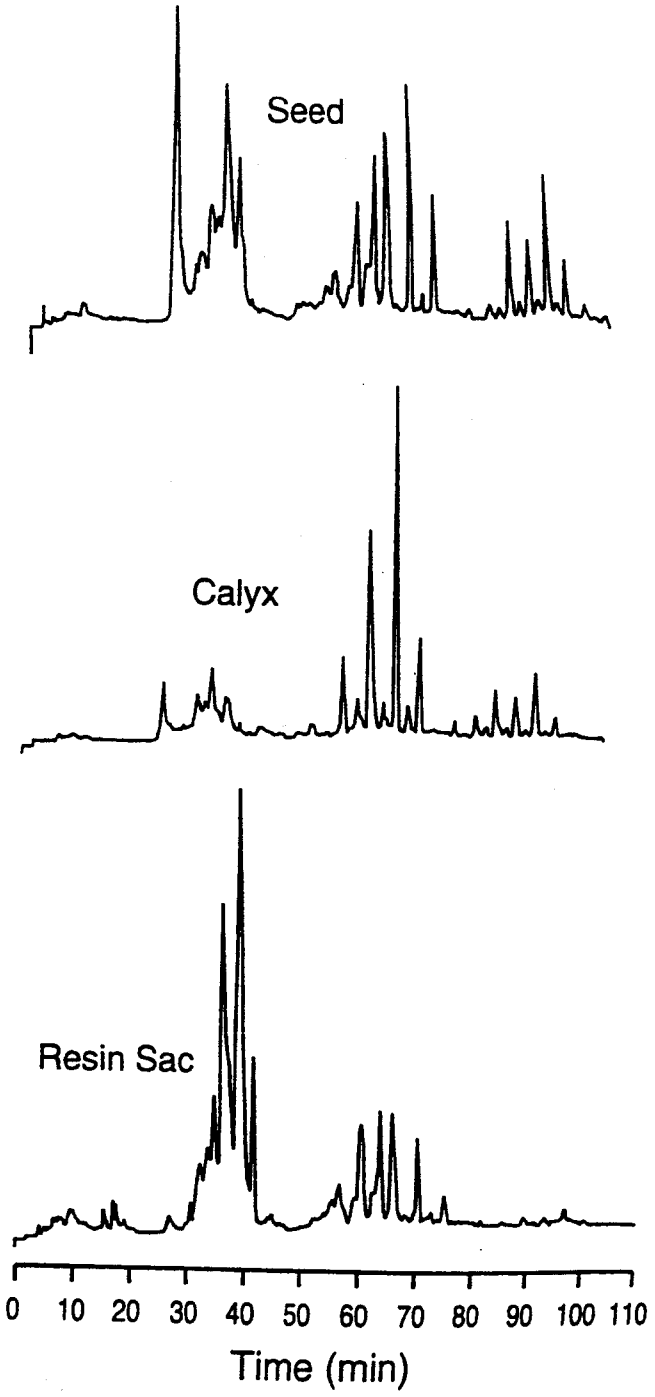

Figure 5 On-line SFE/SFC comparison of dalea spinosa components: whole seed, calyx, resin sac.

of interest are found at retention times that are less than 100 minutes. Grinding the sample introduces heavier components, i.e., glycerides, into the extract which are not desired. Therefore, in this case, extraction on the unground sample is preferred versus sample grinding. This example also illustrates the value of on-line SFE coupled with an appropriate form of chromatography (SFC) for scouting extraction conditions prior to off-line SFE.

II.C. Dispersants, Drying Agents, and the Effect of Water

A frequent problem encountered with SFE involves the extraction of wet (e.g., >5 wt%) samples. The presence of high water content can cause poor contact of the supercritical CO_2 with the sample matrix. In addition, the formation of ice that occurs when the water/CO_2 effluent is depressurized can cause plugging of the flow restrictor. Both problems can be solved by the addition of dispersant/drying agents, either by mixing the sample with the material or by placing the drying agent at the outlet end of the extraction cell. Burford recently characterized a variety of drying agents for their ability to retain water, prevent restrictor plugging, and general SFE performance characteristics (Burford *et al.*, 1993). Several of the drying agents were successful in preventing restrictor plugging, but some analyte losses were reported. For example, some drying agents (alumina and molecular sieve 13X) selectively retained chlorophenols and chloroanilines during SFE from dry samples, while other agents (e.g., Hydromatrix, $MgSO_4$) allowed all of the test compounds to be extracted.

An obvious approach to reducing the water content would be to dry the sample prior to SFE. However, oven or air drying can cause substantial losses of volatile and even semi-volatile organics. For example, when a petroleum waste sludge was air dried at room temperature for 18 hours, 50% of the *n*-tetradecane (b.p. = 252°C) and >99% of *n*-decane (b.p. = 194°C) were lost (Burford *et al.*, 1993). Even the addition of drying agents to such samples should be performed with caution since the addition of many drying agents to wet samples is exothermic. For example, when $MgSO_4$ was added to the same petroleum waste sludge, the losses of *n*-tetradecane and *n*-decane were 30 and 50%, respectively, simply from the heat generated from adding the drying agent (Burford *et al.*, 1993). For this reason, Burford concluded that drying agents are best placed at the outlet end of the extraction vessel, rather than mixed with samples containing volatile components.

As noted above in the section on sample grinding, a smaller sample particle size should theoretically aid in contacting the sample matrix with the extraction fluid, provided that bed channeling effects can be avoided. An alternative to the grinding regime is to use a dispersant that mixes with the sample, thereby accelerating contact with the extraction fluid. Such a moiety must be relatively inert and not permanently adsorb the analytes of interest if direct SFE is to be applied. Considerable success has been achieved with a pelletized diatomaceous earth called Hydromatrix (Hopper *et al.*, 1992) which embraces many of the ideal properties of a dispersant: inertness, a nascent adsorption toward non-polar and moderately polar analytes, and residual hydrophilicity which aids in the retention of modest water levels in moist samples. This medium has been used on food and biological samples (Hopper *et al.*, 1995) and has also been incorporated into environmental analysis (Lopez-Avila *et al.*, 1992).

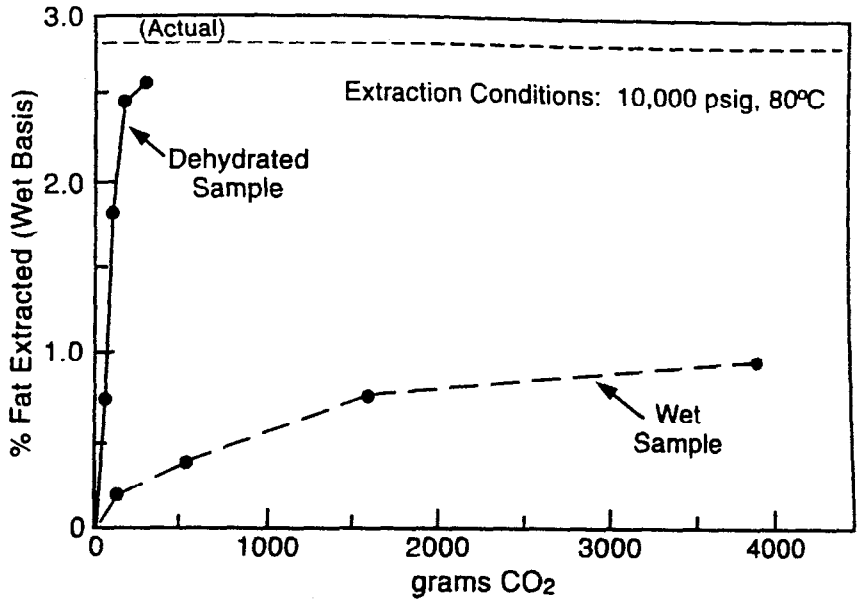

Figure 6 SC-CO_2 extraction of smoked ham sample. Effect of moisture content.

The role of water in SFE can be twofold; that of synergist in facilitating extraction or as an inhibitor in sterically blocking contact between the analyte and the extraction fluid. It has been noted in engineering scale studies of SFE (Peker *et al.*, 1992) that water can modify the morphology of the sample matrix, leading to improved mass transport of the extract (analyte) out of the sample. The most oft-cited case of this phenomena is the extraction of caffeine from coffee beans which can only be effectively accomplished with a moist bean matrix. The natural presence of water in a sample matrix also facilitates its use as an in-situ "cosolvent", since its presence during the extraction of polar analytes frequently leads to enhanced recoveries (McNally, 1995).

Large quantities of water in the sample matrix, which is frequently the case during the SFE of foods, natural products, and biological tissue, can inhibit SFE due to a reduction of contact between the fluid and analytes. Nowhere is this more prevalent than in the extraction of lipids from moist tissue samples. Figure 6 shows the dramatic effect of dehydrating the sample prior to SFE with carbon dioxide on a smoked ham sample containing over 70 wt% water. Quantitative recovery of total fat content (which is desired in toxicant residue analysis) is clearly inhibited by the presence of such a relatively large quantity of water. Gentle dehydration of the sample in an oven prior to SFE rapidly facilitates the removal of fat, reducing both the time and mass of CO_2 required for the extraction.

It should also be appreciated that water has a finite solubility in the primary extraction fluid used in analytical SFE, carbon dioxide. This solubility relationship as a function of

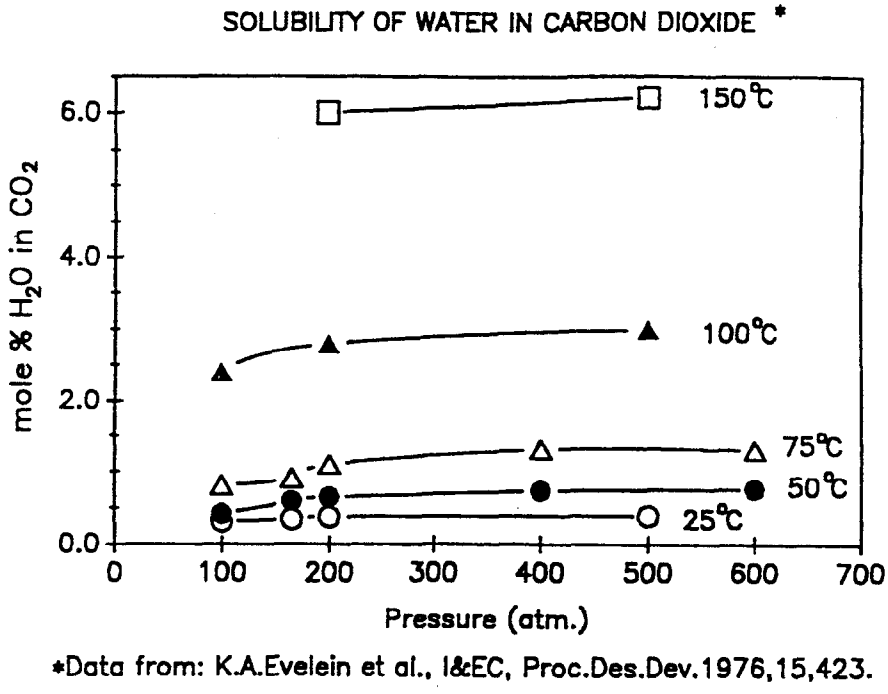

Figure 7 Solubility of water in SC-CO_2.

temperature and pressure is shown graphically for experimental conditions commonly used in analytical SFE in Figure 7. In many cases, control of the water level in the carbon dioxide is desirable, since irreproducible results can be obtained if water from the sample matrix is dissolved into the fluid. A convenient way of suppressing this effect is to add a desiccant to the sample matrix to adsorb the water. Alternatively, a plug of desiccant can also be added to the extraction cell downstream of the sample matrix, but this reduces the ability of the desiccant to disperse and dry the sample matrix.

II.D. Elemental Sulfur

A rather unique problem, restrictor plugging from elemental sulfur, exists for many environmental samples, particularly sediments from marine and related environments where samples frequently contain 2 wt% or more of elemental sulfur. Elemental sulfur extracts very easily in supercritical CO_2 and, in fact, SFE has been used for selective extraction of elemental sulfur from coal (Louie *et al.*, 1993). Unfortunately, elemental sulfur is extremely effective at plugging flow restrictors, and also causes detector problems for many analytical methods (e.g., GC with electron capture detection). Fortunately, the addition of finely divided copper (e.g., two grams of electrolytic grade copper for a five-gram sediment sample) either mixed with the sample or placed at the outlet end of the extraction cell will effectively retain elemental sulfur and provides a simple method to

eliminate restrictor plugging and the presence of elemental sulfur in the SFE extract (Bøwadt *et al.*, 1994a).

III. DEFINING "QUANTITATIVE" RECOVERIES

One of the most vexing problems for SFE (or, for that matter, any other extraction method used for solids and semi-solids) is determining the definition of what constitutes 100% extraction efficiencies. Since the true concentration of analytes can not be known for real (not spiked) samples, 100% recoveries must be defined based on some acceptable criteria. In many cases, the methods used for extraction are historical, and often have not been rigorously tested. For example, one of the most accepted extraction methods, Soxhlet extraction, has been little changed since its first use in 1879. In many cases, the goal of the analyst interested in SFE is to replace an existing method (e.g., to reduce solvent usage or to reduce extraction time), with a desire to obtain results similar to conventional methods, whether or not the conventional method actually yields the "true" value. Therefore, the discussions below are presented to stimulate thought on the part of the analyst new to SFE. As will be seen, the correct definition of "100% recoveries" is certainly a subject of debate, and the proper approach may depend heavily on the characteristics of the sample being extracted as well as on the goals of the analyst.

In essence, the definition of 100% recovery has been based on one or more of three different approaches, i.e., the recovery of spiked analytes, comparison to standard extraction methods (e.g., conventional organic solvent extraction), and the use of multiple extractions.

III.A. Spike and Incurred Analyte Recovery Studies

For environmental solids such as soils, sludges, and sediments, the accepted definition of 100% recovery has traditionally been based on the recovery of known amounts of target analytes spiked onto an appropriate matrix. For example, the organic solvent extraction methods mandated by the U.S. Environmental Protection Agency are considered "quantitative" if the recoveries of spiked analytes (including 2-fluorobiphenyl, 2-fluorophenol, 2,4,6-tribromophenol, and deuterated nitrobenzene, *p*-terphenyl, and phenol) are between ~20 to 140% of the spiked concentrations (U.S. Environmental Protection Agency, 1992). (It is ironic that such a wide range of error is tolerated in the extraction step while much smaller errors are required in the measurement methods used after extraction.) Unfortunately, this approach appears to be even more inaccurate than the 20 to 120% range for many samples, since the extraction rate of spiked analytes can be dramatically faster than analytes that are aged under environmental conditions. This is demonstrated in Figure 8, which shows the SFE recoveries versus time for PAHs (incurred in the sample) and spiked deuterated PAHs (added to the sample) from a petroleum waste sludge (Burford *et al.*, 1993a). Note that the spiked deuterated PAHs always extract faster than their incurred (native) PAH counterparts. If the analyst assumes that 80% recovery of spiked analytes is sufficient to demonstrate quantitative extraction (note that 80% recovery is much more stringent than the EPA requirement), it would be concluded that ~5 to 10 minutes of SFE

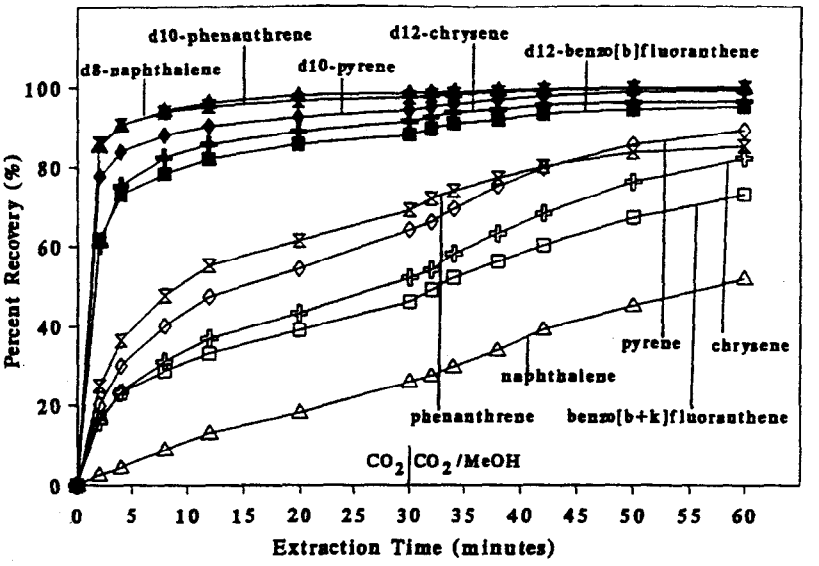

Figure 8 Comparison of SFE extraction rates of incurred (native) PAHs and spiked (deuterated) PAHs from a petroleum waste sludge. The sample was extracted for 30 minutes with pure CO_2 at 406 bar and 60°C, followed by an additional 30 minutes with 10 vol% methanol at the same pressure and temperature. The figure was adapted with permission from Burford *et al.* (1993a).

would be sufficient. However, the results in Figure 8 demonstrate that the actual recoveries of incurred PAHs with a 5 to 10 minute extraction would range from <5% for naphthalene, to only ~25% for the higher molecular weight PAHs. Similar behavior has been demonstrated for the extraction of chlorinated dioxins, PAHs, and PCBs from a variety of environmental samples (Burford *et al.*, 1993a; David *et al.*, 1992; Langenfeld *et al.*, 1992a; Alexandrou *et al.*, 1989), which demonstrates that spike recovery studies are simply not a valid way to determine extraction efficiencies despite the widespread acceptance of this method in environmental analysis. Note that this problem is not unique to SFE, since similar problems have been noted using methylene chloride extraction (Burford *et al.*, 1993a). However, as discussed above, spike recovery studies are extremely important to perform to determine the collection efficiency of the SFE system.

Naturally, confirming the efficacy of SFE on actually incurred analytes in a sample matrix is highly desirable whenever possible. This eliminates any ambiguity with regard to the location of the analytes within the "natural" matrix and takes into account migration of the analytes within the sample matrix as a function of time, or aging of the matrix. It also allows a more accurate assessment to be made of analyte recoveries relative to standard solvent extraction methods, or can show the superiority of SFE relative to such techniques or methods as documented in the literature (Hawthorne *et al.*, 1994). An example of this trend is noted in Figure 9 where equivalent results were achieved on three of four different kinds of poultry tissues containing incurred organochlorine pesticide residues. Such residues were developed during feeding studies of chicken flocks fed both spiked and unspiked rations. However, there is a pronounced difference in the results between the organic

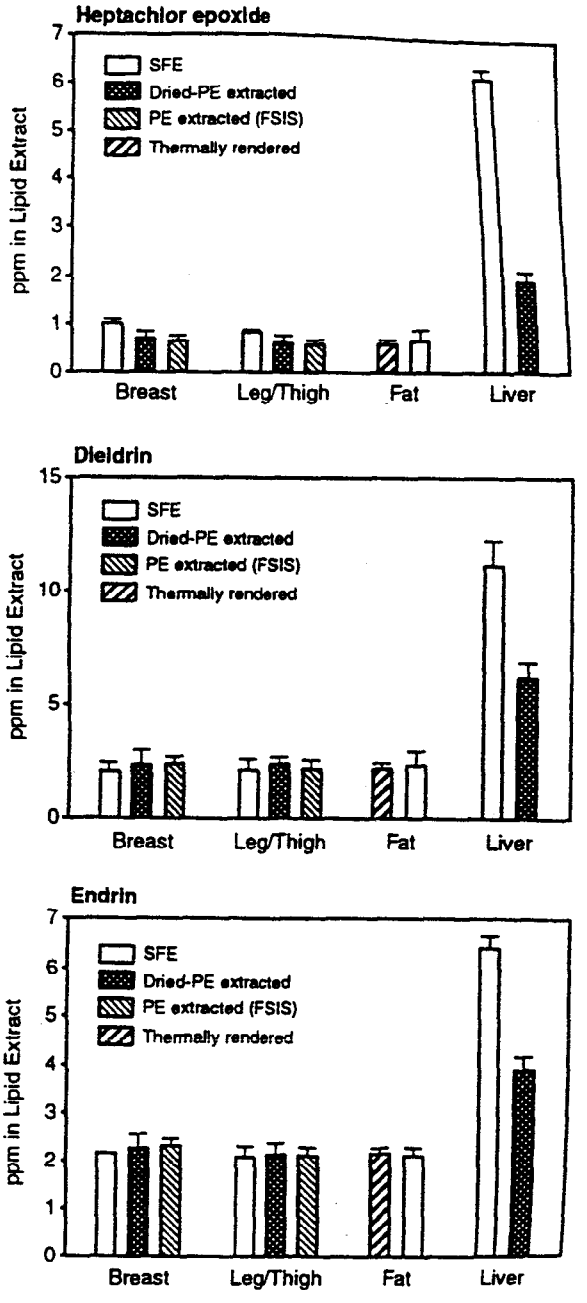

Figure 9 Comparison of extraction methods on poultry tissues from the same chicken. Standard deviation is indicated by marks on the top of each bar. Prior to the petroleum ether extractions (PE), tissue samples were oven dried to <5 wt% water (dried PE extracted) or by mixing with Na_2SO_4 (FSIS) according to the Food Safety and Inspection Service (U.S. Department of Agriculture). Peritoneal fat samples were thermally rendered at 80°C. Details of the procedures are in Snyder *et al.* (1993).

Table 3 % Pesticide recoveries from wheat at 0.1 ppm fortification level.

Conditions: 345 bar, 100 l CO_2 (Expanded)	*40°C*		*60°C*		*80°C*	
	Run 1	*Run 2*	*Run 1*	*Run 2*	*Run 1*	*Run 2*
Dimethoate	88	87	82	101	77	84
Methyl Parathion	89	89	92	103	91	93
Pirimiphos Methyl	96	95	101	108	99	100
Chlorpyrifos	97	97	105	113	99	101
Malathion	93	95	102	109	96	97
Dieldrin	95	91	104	104	93	91
Methoxychlor	94	94	85	107	97	103
Carbofuran	89	97	97	98	92	95
			Incurred Residue Results (ppm)			
Methyl Chlorpyrifos	0.039	0.038	0.042	0.043	0.044	0.041

solvent extraction methods and that obtained via SFE on liver tissue. This trend was found repeatable from livers taken from several chickens and seems to confirm the superiority of SFE for extracting the pesticide residues from this difficult-to-extract biological tissue. This is consistent with the theoretical view that diffusion of supercritical fluids into such sample matrices is superior to that obtainable with conventional liquid extractants. That SFE is capable of reproducibly extracting incurred residues is borne out by the results shown in Table 3. Here the detection of an incurred residue of methyl chlorpyrifos occurred during SFE studies on a wheat matrix spiked with eight different pesticides. SFE consistently yielded high recoveries on this incurred residue, leading further credence to SFE as a viable technique for trace residue analysis.

III.B. Multiple Sequential Extractions

An alternate approach to determining extraction efficiencies which was used in early SFE methods development was to compare the quantities of analyte extracted during a first time fraction (e.g., 30 minutes) versus a second SFE of the sample conducted under the same conditions. For this approach to be valid, the individual molecules of a particular analyte must all extract at the same approximate rate. However, extraction profiles frequently show an initial fast extraction followed by a slow extraction period (discussed in more detail later in the text). In addition, it is common for one SFE condition to extract only a fraction of a target analyte in (e.g.) 30 minutes, but very little more of that analyte even after much longer additional extraction times if the SFE condition is left constant (Bøwadt *et al.*, 1995; Bartle *et al.*, 1990). This is demonstrated in Figure 10 by the extraction of a PCB congener from river sediment. Note that if the amount extracted at 50°C for the first 20 minutes is compared to the next 20 minutes, it could easily be concluded that the extraction is fairly complete (i.e., there was very little extracted in the second 20-minute fraction). However, when a stronger extraction condition is used (in this case, raising the temperature to 200°C while maintaining the pressure at 640 bar), the amount of the PCB extracted increases

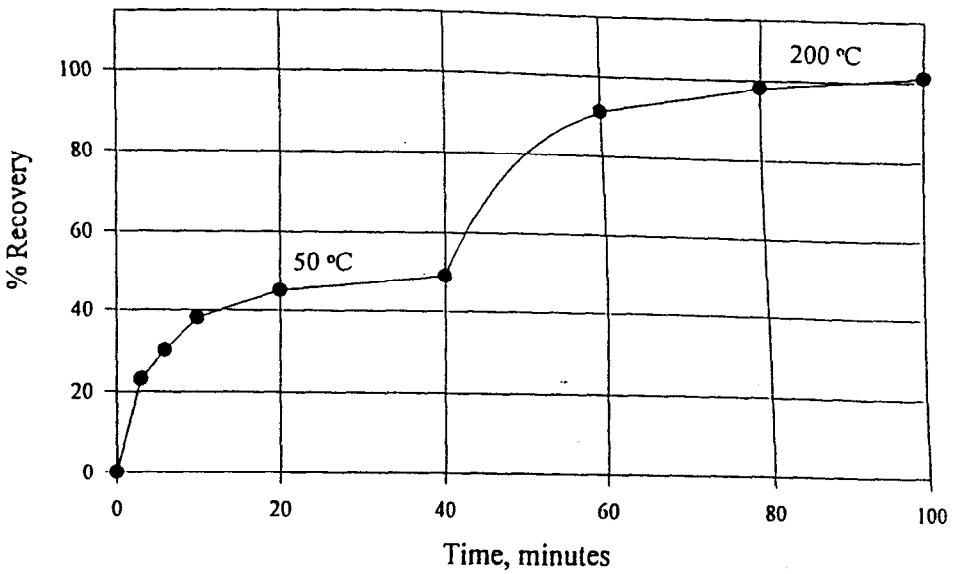

Figure 10 Extraction of PCB 52 from a certified river sediment (NIST SRM 1649) using pure CO_2 at 641 bar at 50°C for 40 minutes followed by extraction at the same pressure and 200°C for an additional 60 minutes. The figure was adapted with permission from Bøwadt *et al.* (1995).

when a stronger extraction condition is used (in this case, raising the temperature to 200°C while maintaining the pressure at 640 bar), the amount of the PCB extracted increases dramatically, and 100% recovery is achieved (compared to the certified value based on Soxhlet extraction).

While multiple extractions at the same condition are not generally valid to determine extraction efficiencies, performing extractions with SFE followed by an independent method (e.g., organic solvent extraction) appears to be a valid method of determining whether "100% recovery" has actually been achieved. The assumption here is that if no significant analyte is found in the organic solvent extract performed on the residue of the SFE extract, than the SFE extraction was quantitatively efficient (Burford *et al.*, 1993a; Hawthorne *et al.*, 1993).

III.C. Comparison to Standard Methods

Since the analyst's goal frequently is to obtain extraction results that compare with conventional extraction methods historically used in their laboratory, the definition of 100% recovery is logically based on comparing the SFE results to the conventional method. For many samples, this comparison can be validly based on Soxhlet extraction, both because of its wide-spread use in environmental and biological material extractions, as well as because it is an effective extraction method. (It should be noted, however, that Soxhlet extraction is not necessarily correct as evidenced by an increasing number of

Table 4 Variation in 7 total fat determinations (gravimetric assay) for a ground beef sample using different SFE and sample prep conditions.

Conditions: 379 bar, 80°C, 45 min extraction time *Solvent/Sample Prep Conditions*	*Weight % Fat*
$SC\text{-}CO_2$/Neat	14.74
	15.45
	15.39
	15.36
$SC\text{-}CO_2$/Dehydrated	15.53
	14.67
	14.92
$SC\text{-}CO_2$/Acid Hydrolyzed	15.84
	22.50
	17.97
	16.09
	15.48
$SC\text{-}CO_2$–5% EtOH/Neat	21.95
$SC\text{-}CO_2$–5%$HCCl_3$/Neat	17.19
$SC\text{-}CO_2$–5% MeOH + 5%$HCCl_3$/Neat	20.95
$SC\text{-}CO_2$/Acid Hydrolyzed/FAME	14.89

is certainly an appropriate approach which has the additional advantage that these materials are available to any research group wishing to compare results with other laboratories. However, one caution should be used when certified reference materials are used, i.e., it is important to ensure that the methods used to analyze the extracts are exactly comparable to those used for the certification (e.g., identical standards and chromatographic conditions) to ensure that differences between the SFE extracts and the certified values are a result of *extraction* rather than *analysis* conditions. To eliminate this problem, we frequently perform Soxhlet extractions and SFE on parallel samples and analyze the extracts under identical conditions to ensure that differences are indeed based on differences in extraction efficiencies.

Another example of the danger in comparing SFE results with those obtained by established methods is in the determination of fat content of foods. Again, Soxhlet-based techniques have been used extensively for many years employing a variety of extraction solvents and sample preparation methods, and not surprisingly, this has led to a multiplicity of values for the "true" fat content of a particular matrix. This disagreement is not so pronounced for matrices containing lipid moieties that have been subjected to refining, processing, or compounding of fat from purified ingredients, but serious discrepancies have arisen particularly in the area of biological matrices where the lipid species are so diverse and frequently associated with carbohydrate or protein matter. Newer methods and definitions of fat (Fed. Regist, 1993) have eliminated much of the ambiguity today, and permitted SFE researchers to couple SFE with the new Nutritional Labeling and Education Act (NLEA) protocols for speciated fat in food products (House *et al.*, 1994; Carpenter *et al.*, 1993). Nonetheless, gravimetric-based SFE methods for fat determination can be susceptible to error just as in the above classical methodology. As shown in Table 4 for the SFE determination of fat content in a ground beef sample, the state of the sample

(hydrated vs. dehydrated), sample preparation, use of cosolvents, and method of analysis all impact on the final fat result obtained under identical SFE conditions. Much of this confusion can be eliminated by using SFE in conjunction with an analyte specific assay (fatty acid methyl ester analysis), as has been shown by King and his colleagues (King *et al.*, 1996; Snyder *et al.*, 1996).

IV. SIMPLE TWO-STEP SFE MODEL

The analyst should now (hopefully) understand how to collect extracted analytes, which (if any) sample pre-treatment may be needed, and which definition of 100% recovery is to be used. The so-called "mystery" of SFE methods development can now largely be removed by a simple understanding of SFE processes. A more in-depth discussion of SFE mechanisms is given in the Chapter by Bartle and in other references (Bartle *et al.*, 1990, 1992; Clifford *et al.*, 1995; Langenfeld *et al.*, 1995; Pawliszyn, 1993; Hawthorne *et al.*, 1995). However, it is clear that additional research is needed to fully understand the SFE process, mostly because of a lack of true understanding of the interaction of analytes with their matrices. Nevertheless, the simple two-step model described below does much to describe the factors needed for quantitative SFE.

In order to move the target analytes from the sample matrix to the collection device two steps must occur, either of which (or both) can control the extraction process. First, the analyte must move from the active site in (or on) the sample into the extraction fluid (Step 1). Second, the analyte must be eluted from the extraction cell in a process analogous to frontal elution chromatography (Step 2). Step 1 has been termed the "desorption/kinetic" process (or "initial removal" process), and requires that the analyte leave its sorption site and be transported to the bulk extraction fluid. This process can be thought of as occurring once for each analyte molecule, i.e., once the molecule is removed from its initial binding site the extraction is controlled by Step 2. The driving force for Step 1 is the concentration gradient which exists between analytes in (or on) the matrix, and analytes in the bulk extraction fluid, as well as the ability of the SFE conditions to displace the analyte from the initial active sites. Therefore, the extraction of samples limited by Step 1 can be enhanced by increasing the rate of this initial removal process using methods which will be discussed for the remainder of this chapter.

Step 2 can be termed as the "solubility/elution" process, and is controlled by the same factors which control chromatographic retention, i.e., compounds with high solubility will elute more rapidly, as will compounds with little matrix interaction (in terms of chromatographic partitioning to the matrix). Thus, while Step 1 can be viewed as irreversible (occurs once during an extraction) Step 2 can be viewed as a reversible process (analogous to chromatography). Therefore, extractions limited by Step 2 can be enhanced by the same types of approaches that accelerate chromatographic elution as will be discussed in subsequent sections.

While both Step 1 and Step 2 can be important for SFE of a particular sample, one step or the other frequently is more important. Since Step 1 is best described as a kinetic process controlling the rate at which a molecule moves from its initial active site to the bulk extraction fluid (as described by the "hot-ball" model for SFE described by Clifford

et al.) (Bartle *et al.*, 1990, 1992; Clifford *et al.*, 1995), and Step 2 is best described as a chromatographic process, a simple method to determine the relative importance of Step 1 is to determine the effect of SFE flow rates on the extraction rates of target analytes (Hawthorne *et al.*, 1995). As will be described, the results of a simple flow rate study can be used to categorize samples into those under Step 1 and/or Step 2 control, and can also provide a basis for methods development.

V. EFFECT OF SFE FLOW RATE AND EXTRACTION MODE

V.A. Determining Extraction Mechanism by Effect of Flow Rate

Samples controlled by Step 1 (desorption/kinetic) are limited by the rate at which the analyte molecules move from the initial active sites to the bulk fluid much more than they are limited by solubility or *re*-partitioning to matrix active sites after they are once solvated. Therefore, Step 1-controlled samples will show little (if any) dependence on the flow rate used for SFE as long as the void volume of the SFE cell is swept every few minutes. In contrast, samples controlled by Step 2 (solubility/elution) are controlled by chromatographic considerations; thus, increasing the SFE flow rate will proportionally increase the extraction rate.

The concept of invoking a chromatographic process to explain SFE from a packed cell bed is not new and has been developed by several investigators applying SFE for chemical or food engineering purposes. For example, Lee *et al.* (1986) modeling the extraction of canola seed from a tubular extractor using chromatographic principles, while Goodrum *et al.* (1989) demonstrated the analogy between oil elution from peanut seed beds and frontal chromatography profiles under subcritical conditions with carbon dioxide as a solvent. This has been confirmed by one of the authors for elution of soybean oil from a packed bed of flakes in a tubular extraction vessel. Pawliszyn (1993) has used similar chromatographic models in modeling analytical scale SFEs.

Since it would seem intuitive that higher flow rates would yield faster extraction rates, examples of samples controlled by Step 2 will be discussed first. A classic example of a sample controlled by Step 2 is one that has very high concentrations of analytes, and is, therefore, controlled by the ability of the fluid to solvate the analytes. An example of such an extraction is shown in Figure 11 by the extraction of fat from potato chips. When the extraction flow rate is doubled (e.g., from 0.7 to 1.4 ml/min), the extraction rate of the fat is also doubled. Thus, this extraction is controlled by Step 2, and Step 1 (e.g., the kinetics of the solvation process) is fast. For such samples, the "% recovery" versus "extraction time" plots are nearly linear until the approach to 100% recovery. Also, for such samples the *volume* of extraction fluid can be used to define a quantitative extraction condition, since the recovery is directly related to the volume used, while the *time* of extraction is not important, as long as the sample is swept with sufficient volumes of the extraction fluid (which is, in essence, saying that the extraction depends directly on flow rate). Similar results would be expected for analytes (e.g., fat-soluble pesticides) which extract with the fat. This is confirmed by a plot of the inverse relationship (amount of target analyte remaining in the sample matrix) as shown in Figure 12. Here both chlorinated

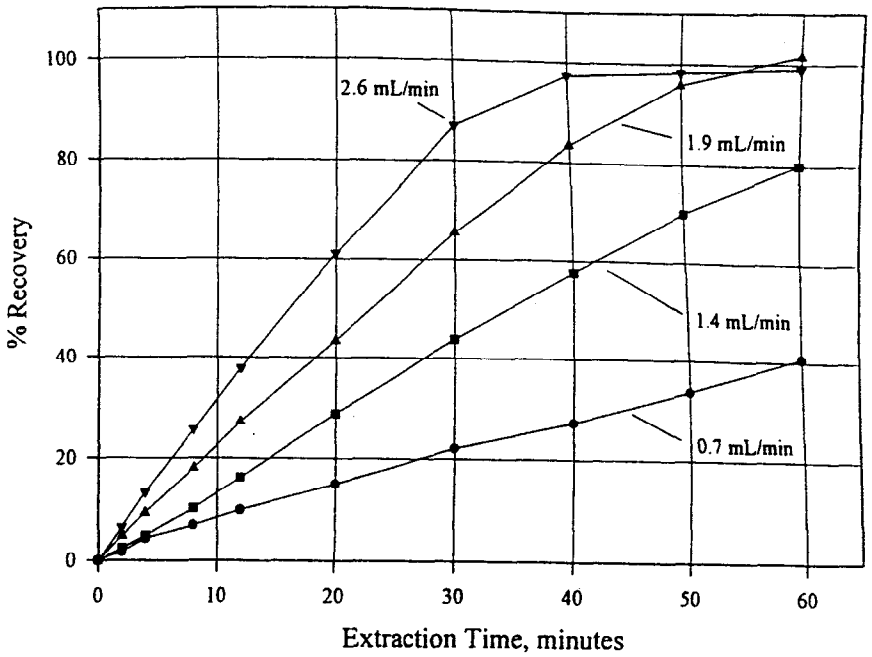

Figure 11 Effect of CO_2 flow rate (measured at the pump as compressed CO_2) on the extraction of fat from potato chips at 345 bar and 60°C. The figure was adapted with permission from Hawthorne *et al.* (1995).

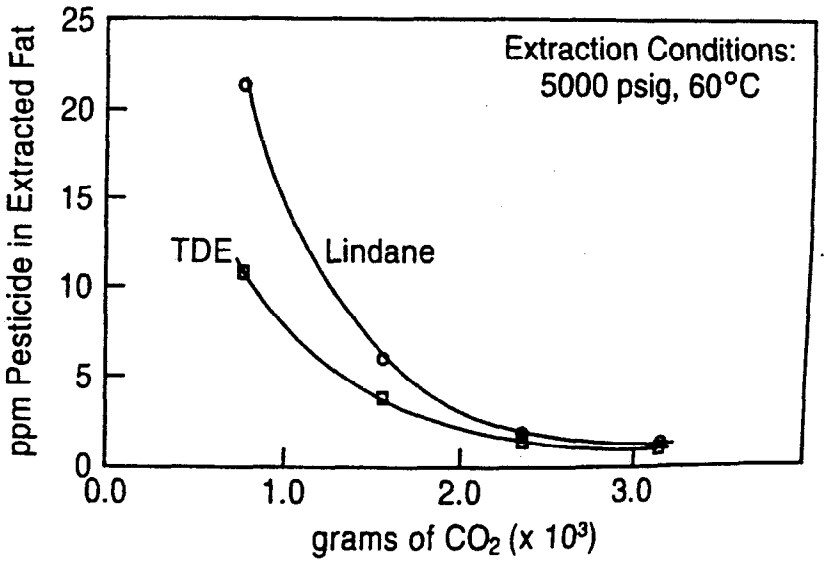

Figure 12 Rate of pesticide removal during fat extraction.

pesticides, TDE and lindane, follow a similar locus as the extraction of fat, approaching an asymptotic limit as the SFE continues.

In contrast to the fat extraction, the extraction of many samples is controlled more by Step 1 than Step 2. Since the major factor limiting such extractions is the initial removal of the analyte from the matrix into the extraction fluid, and is not the subsequent chromatographic elution, such extractions will show little dependence on SFE flow rate. As shown in Figure 13 by the extraction of *m*- and *p*-xylene from polystyrene beads, virtually no change in extraction rate is observed when the flow rate is increased from 0.7 to 1.25 ml/min. This would be expected since the rate-limiting step for this extraction is the diffusion of the analyte from the interior to the surface of the beads (a Step 1 process described by the "hot-ball" model) (Bartle *et al.*, 1990), and not the solubility/elution process. Similarly, the extraction of limonene from lemon peel (Figure 13) shows no dependence on flow rate, again demonstrating that the kinetics of the Step 1 process are slow compared to chromatographic retention or solubility problems that would cause Step 2 to predominate.

It is extremely important to note that the controlling step may be different for the same analytes extracted from different matrices. For example, Figure 14 shows the extraction of the PAHs, benzo[*b*]- and benzo[*k*]fluoranthene from a highly contaminated soil and a low-level contaminated soil. For the highly-contaminated soil, the PAH extractions showed a strong dependence on flow rate (Step 2 controlled), at least until higher flow rates were reached. In contrast, when the same PAHs were extracted from the low-level contaminated soil, there was no significant dependence on the CO_2 flow rate (Step 1 controlled).

V.B. Effect of Sample Size

The same considerations for determining the importance of Step 1 and Step 2 for a particular sample using flow rate apply to sample size. That is, the % recovery versus time plots for samples that are controlled by Step 2 (dependent on flow rate) will show slower extractions if the sample size is increased (with no change in flow rate). Similarly, samples controlled by Step 1 will show the same extraction rate, regardless of sample size (as long as the flow rate is sufficient to sweep the sample void volume every few minutes). This is demonstrated in Figure 15 by the PAH extractions for the same highly-contaminated and low-level contaminated soils. The highly-contaminated sample (controlled by Step 2) shows a marked reduction in extraction rate (in terms of % recovery versus time) which would be expected since this extraction is controlled by the solubility/elution step. (Note that the mass of PAHs extracted per unit time was similar, regardless of the sample size, but the % recovery versus time was much lower for the larger sample.)

In contrast, the extraction of the low-level contaminated soil (controlled by Step 1) showed no significant difference in extraction rate (% recovery versus time). This behavior would be expected since the extraction of this sample is controlled by the rate of the desorption/kinetic step. (Note that the mass of PAHs extracted per minute from the 0.5-gram sample would be ~eight times less than from the 4-gram sample, since the % recovery versus time plots are similar.)

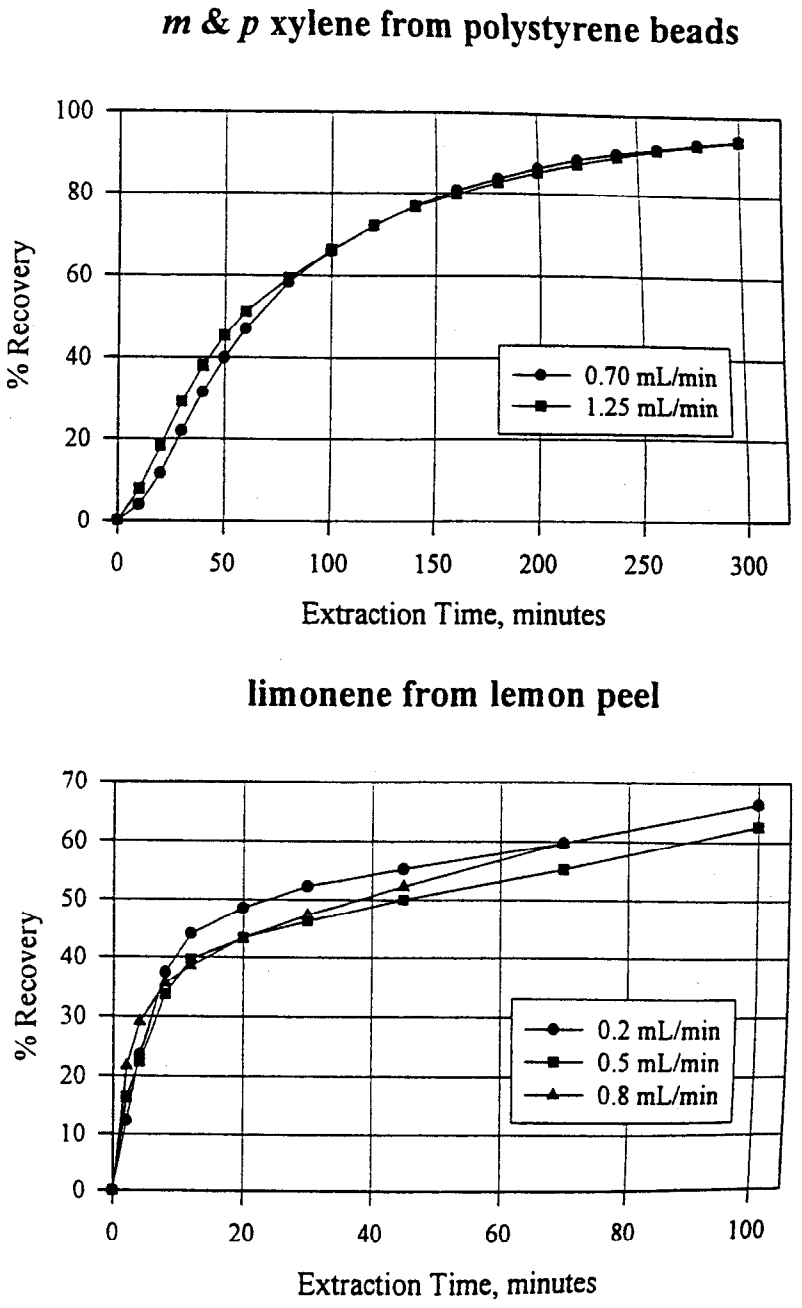

Figure 13 Effect of CO_2 flow rate on the extraction of *m*- and *p*-xylene from polystyrene beads at 405 bar and 45°C (top) and on the extraction of limonene from fresh lemon peel at 405 bar and 50°C (bottom). The figure was adapted with permission from Hawthorne *et al.* (1995).

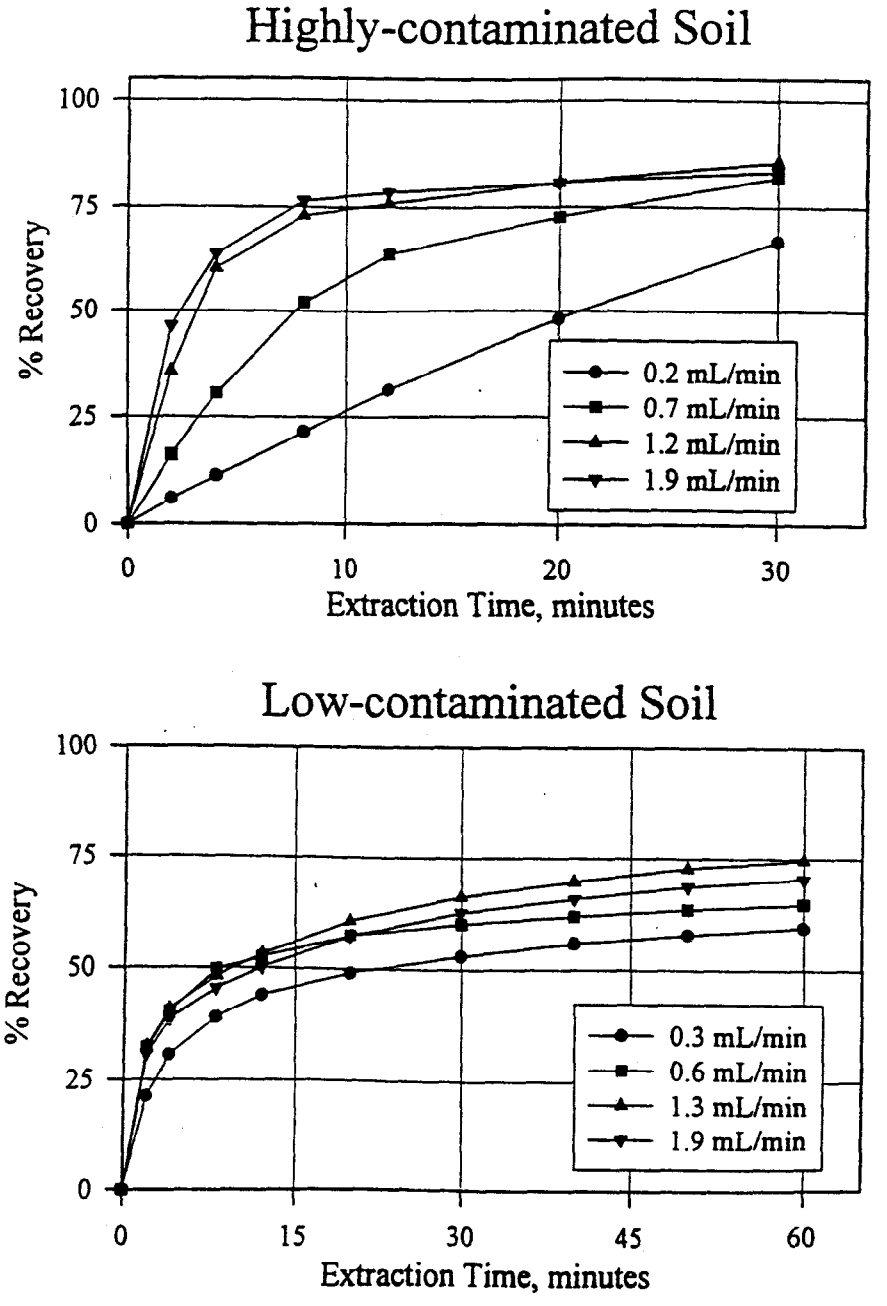

Figure 14 Effect of CO_2 flow rate on the extraction of benzo[*b*]- and benzo[*k*]fluoranthene from a highly contaminated soil and from a lower-contaminated soil. Extractions were performed with pure CO_2 at 405 bar and 60°C as described in Hawthorne *et al.* (1995).

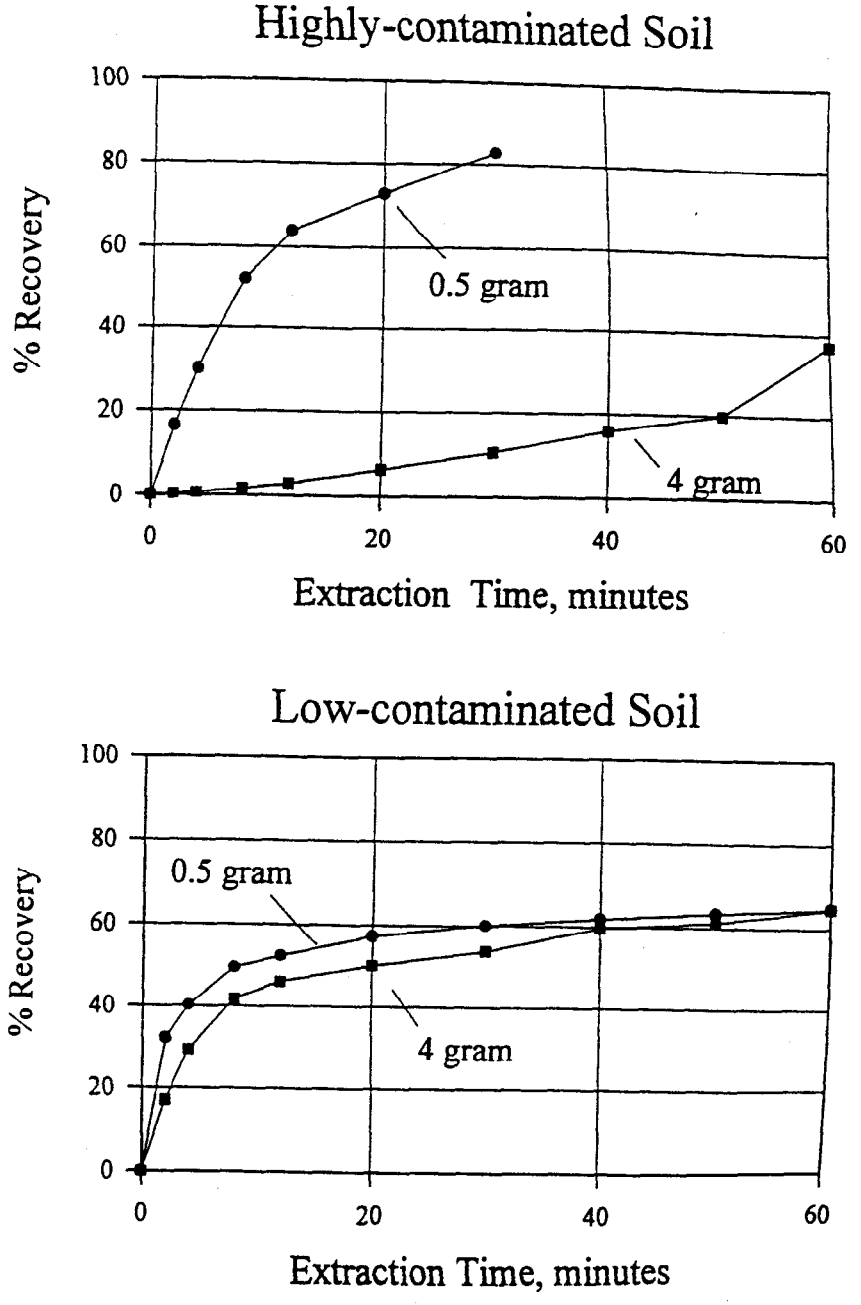

Figure 15 Effect of sample size on the extraction of benzo[*b*]- and benzo[*k*]fluoranthene from a highly contaminated soil and from a lower-contaminated soil. Both samples were extracted with pure CO_2 at 405 bar and 60°C at a constant flow rate of 0.6 to 0.7 m/minute of compressed CO_2 as described in Hawthorne *et al.* (1995).

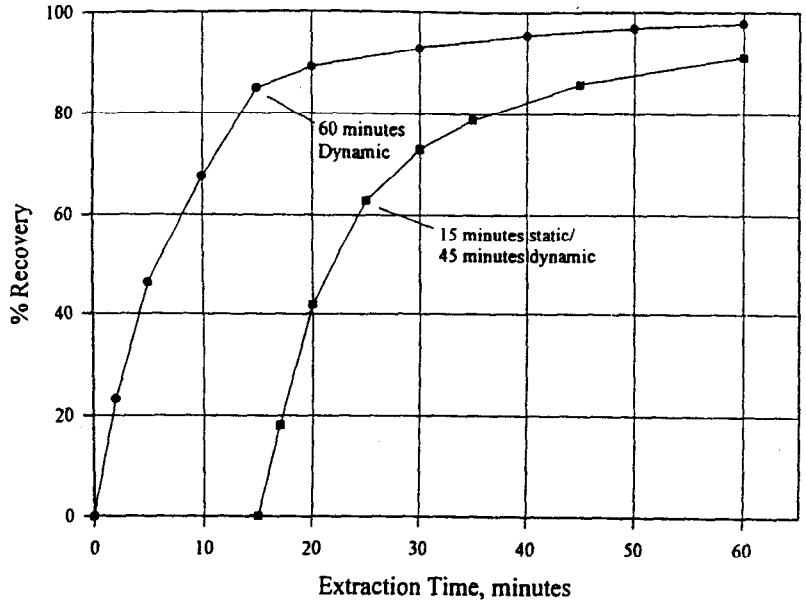

Figure 16 Effect of static versus dynamic SFE on the extraction of motor oil from soil using pure CO_2 at 405 bar and 70°C. The static step was for 15-minutes followed by 45 minutes of dynamic extraction at 0.8 ml/minute. The dynamic extraction was performed with constant flow at 0.8 ml/minute. The figure was adapted with permission from Hawthorne *et al.* (1995).

versus time plots are similar.)

V.C. Static versus Dynamic SFE

The effect of flow rate on the extraction rate studies just described also relate to the relative abilities of static and dynamic SFE to yield effective recoveries. For samples controlled by Step 1, a static SFE step will be nearly as effective as a dynamic step, since the rate-controlling process is the initial desorption/kinetic step. (This is, of course, assuming that a sufficient dynamic step is used after the static step to sweep the extracted analytes from the sample.) For example, the soil contaminated with lower levels of PAHs discussed above shows identical recoveries using 30 minutes of dynamic extraction or 15 minutes of static followed by 15 minutes of dynamic extraction (Hawthorne *et al.*, 1995). In contrast, samples controlled by Step 2 will not be effectively extracted by a static step because the solubility/elution step depends on the volume of CO_2 passing through the sample. This is demonstrated in Figure 16 by a comparison of the extraction rate of a soil contaminated with a large amount (~29 mg/g) of used motor oil. Since this sample is controlled by the Step 2 solubility/elution step, the use of a 15-minute static step before dynamic SFE is not effective, and simply delays the recovery of the motor oil by ~15 minutes as shown in Figure 16. Similar considerations apply to the use of modifiers added to the sample versus mixed with the extraction fluid as discussed below.

usually affected by injecting the co-solvent into the extraction cell or, in one of the authors' laboratories, by inserting a saturated plug of glass wool ahead of the sample in the extraction cell. The static hold step with the addition of a co-solvent facilitates the mass transport of the co-solvent into the sample matrix allowing the co-solvent to solvate or displace the analyte from the sample before initiating a dynamic sweep of the cell. This has been used to good effect by Langenfeld *et al.* (1994), Hawthorne (1993a) and Yang *et al.* (1995), and by France *et al.* (1991) when coupling SFE with immunoassay detection.

V.D. Implications of the Flow Rate Experiment for Methods Development

The simple flow rate experiment described above can be used to support methods development when initial SFE conditions fail to give adequate recoveries. Some general comments on developing SFE methods based on whether a sample extraction is primarily limited by the desorption/kinetic step or the solubility/elution step are given below (Hawthorne *et al.*, 1995):

Step 2-controlled samples

Samples where extraction rates are controlled primarily by the solubility/elution step tend to have high concentrations of analytes that are only weakly associated with the sample matrix. The following statements apply to such samples:

1. Faster flow rates will yield higher extraction rates. Therefore, flow rates should be increased if experimentally convenient. It is especially important to determine if higher flow rates still yield good collection efficiencies, since too high of a CO_2 flow rate will lower collection efficiencies because of the large volumes of expanding gaseous CO_2. Increasing extraction time using the same flow rate will also be effective.
2. For such samples, the extraction efficiency depends on the total volume of extraction fluid used (rather than the time used for extraction). Therefore, methods can be defined based on using a certain volume of extraction fluid for each sample.
3. Smaller samples will extract more rapidly than larger samples (assuming the same flow rate).
4. Static extraction steps will be much less effective than the same time used for dynamic extraction.
5. Efforts should be made to increase analyte solubility by increasing pressure, increasing or decreasing temperature (note that higher temperatures at a constant pressure can either increase or decrease solubilities, depending on the analyte vapor pressure (Bartle *et al.*, 1991; Miller *et al.*, 1995), by changing the extraction fluid, or adding an organic modifier which is chosen to increase analyte solubility and/or decrease the equilibrium adsorption to matrix active sites.

Step 1-controlled samples

Samples where the extraction rate is controlled primarily by the rate of the initial desorption/kinetic step tend to have lower concentrations of analytes, with a higher proportion of the

analytes experiencing strong analyte/matrix interactions. For such samples the following statements apply:

1. SFE flow rate has little or no effect on the extraction rate as long as the void volume of the sample is sufficiently swept during the extraction. Increasing extraction time beyond 30 to 60 minutes becomes increasingly less effective for such samples because the extraction rate tends to drop during the extraction.
2. For such samples, the extraction efficiency depends primarily on the total time used for the extraction (rather than the volume of fluid used for extraction). Therefore, methods cannot be adequately defined based on the total volume of fluid used, but must be based on the time used for extraction.
3. Sample size has little or no effect on extraction rate (as long as the void volume is sufficiently swept during the extraction).
4. Static extraction steps are often as effective as dynamic extraction performed for the same period of time, as long as the subsequent dynamic extraction step is sufficient to sweep the sample void volume.
5. Improving extraction efficiencies should be based on considering matrix/analyte interactions more than analyte solubility. Effective approaches for samples limited by the desorption/kinetic process include grinding the sample (for samples such as polymers where the analytes are limited by diffusion through the sample matrix), adding a modifier to disrupt analyte/matrix interactions (rather than to increase analyte solubility), and increasing extraction temperature to increase the rate of the desorption/kinetic process.

VI. FLUID CHOICE AND MODIFIERS

VI.A. Pure Fluids

The characteristics of different fluids used for SFE of environmental samples have recently been reviewed (Bøwadt *et al.*, 1995), and will only briefly be summarized here. For practical reasons of low cost, reasonable critical parameters (T_c = 32°C, P_c = 74 bar), low toxicity, and ease of removal from the extract, CO_2 has been used for nearly all applications of SFE. The major drawback of CO_2 is its lack of polarity. In a few cases (e.g., the extraction of chlorinated dioxins from fly ash (Onuska *et al.*, 1991; Alexandrou *et al.*, 1992)), N_2O has been more successful at displacing analytes from matrix active sites and yielded higher recoveries than CO_2. Unfortunately, N_2O can cause violent explosions with samples having significant oxidizable material, and its use should be avoided (Raynie, 1993). Some Freons, especially $CHClF_2$ have shown excellent extraction efficiencies when CO_2 fails to yield high recoveries (Hawthorne *et al.*, 1992a). Unfortunately, the use of halogenated fluids is objectionable because of their environmental impact.

One practical aspect of choosing a fluid to use for SFE is its purity level. Early studies in SFE showed that even for the most popular extraction fluid, CO_2, that available analytical-grade gases were deficient. The high purity requirements for analytical SFE originate from several sources. A key factor is that impurities in the extraction fluid are

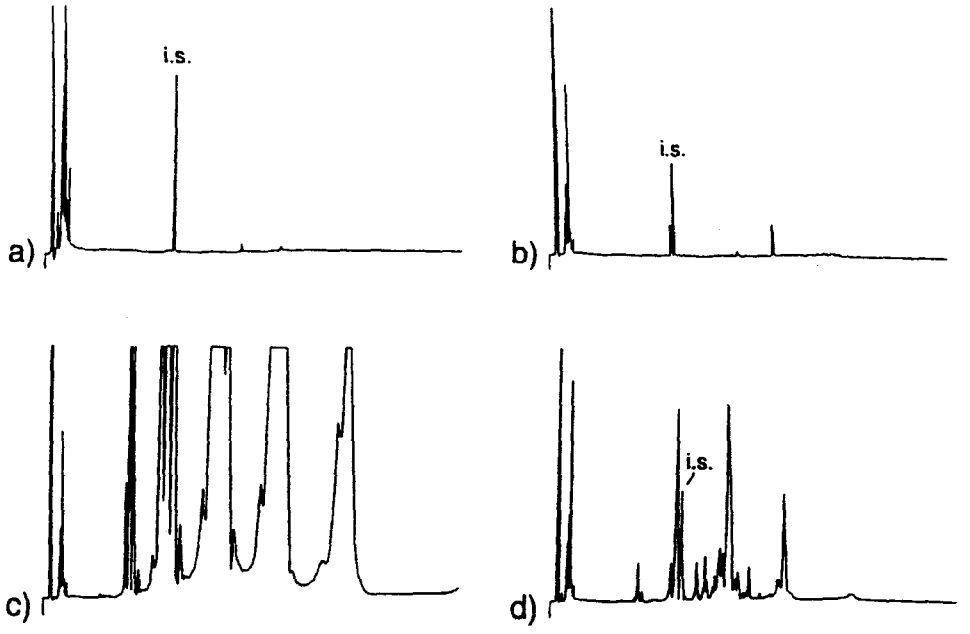

Figure 17 Capillary GC/ECD chromatograms of various carbon dioxide gas grades: (a) SFE-SFC, (b) food, (c) welding, and (d) SFC.

concentrated in the collection vessel. Although gases have not been traditionally thought of as extraction solvents until the advent of analytical SFE, these concentrated impurities can present problems if they are contained in extracts being analyzed by the normal array of analytical detection schemes. For example, if one is concerned with electron capture detection of pesticides, then the extraction fluid must contribute minimal impurities to the final extract or these could interfere in the final gas chromatographic analysis. This is nicely illustrated in Figure 17 where the GC/ECD profiles have been run on equal amounts of a pure fluid extract (no sample) using the grades of carbon dioxide specified. Clearly, SFE-SFC and food grade carbon dioxide are superior to the welding grade and SFC-grade fluids with respect to using electron capture detection. This of course can change depending on the terminal analytical detection method that is employed (fluorescent, ultraviolet, etc.). Today, highly purified CO_2 is available at a premium price. Alternatively, home-made cleanup traps can be fashioned for use with more contaminated fluid sources which will reduce impurities to suitable levels for ultra trace analysis. These have consisted of activated aluminum oxide or mixtures of this sorbent with activated carbon or molecular sieve (Lopez-Avila *et al.*, 1992). Unfortunately this requires the analyst to change traps at regular intervals to avoid contaminant breakthrough due to the sorbent becoming saturated with impurities.

Although CO_2 is by far the most commonly used extraction fluid for the reasons noted above, there are several other candidates that have utility also, or niche applications. Of particular note are the fluorocarbons (those not banned by environmental considerations),

SF_6, and fluoroform. Levy *et al.* (1989) has shown that SF_6 under appropriate conditions can selectively extract alkanes with respect to aromatic hydrocarbons. Similarly, the hydrogen bonding propensity of fluoroform (HCF_3) allows differential extractions to be accomplished on polar analytes, such as opium alkaloids. Stahl *et al.* (1980) demonstrated that the alkaloid, thebaine, is preferentially solubilized over codeine and morphine by fluoroform. Fluoroform also has a low propensity for fat (Stahl *et al.*, 1988) which makes it attractive for extracting analytes from lipid-rich matrices. This property has been exploited by Taylor and King (1994b) to selectively extract pesticides from poultry fat as shown in Figure 18, thereby avoiding traditional time-consuming cleanup techniques. More recently Taylor and coworkers (Ashraf-Khorasanni *et al.*, 1996) have used the selective extraction properties fluoroform to extract drug moieties devoid of extraneous lipid coextractives.

In recent years, helium has been introduced into cylinders of compressed fluids such as CO_2 in order to allow pumping of the fluid in the liquid state, to avoid the installation of a cooling bath to liquefy the fluid at the pump head. This, unfortunately, affects the solubility of analytes in SC-CO_2 due to the dissolution of a small quantity of helium in the liquefied CO_2 (King *et al.*, 1995). Therefore, SFE results can be affected by this factor, particularly if the extraction time or quantity of fluid used is not in generous excess. An example of this effect is shown in Figure 19 for the solubility of a model fatty substance, soybean oil, in SC-CO_2 as a function of pressure. Note that the presence of helium in the feed cylinder reduces the oil solubility in SC-CO_2 by over 50% at 5000 psi and approximately 30% at 689 bar, relative to either gaseous or liquid CO_2 feeds. This can have a significant effect on the design of extraction conditions for the extraction of fatty substances from foods and biological tissue.

It must be noted that the two authors have different (and contrary) experimental evidence on the effects of using CO_2 with helium head pressure on SFE results. In contrast to the effects of added helium on soybean oil as just discussed, determinations of cholesterol solubility performed with and without helium present in the CO_2 gave the same values. In addition, the extraction rate of cholesterol from egg powder was the same whether or not CO_2 with helium headspace or pure CO_2 was used (Hawthorne *et al.*, unpublished results). It should be further noted that all extractions of environmental samples presented in this chapter are based on experiments using CO_2 with helium headspace.

VI.B. Modifiers

Since CO_2 is relatively non-polar, it has a limited ability to dissolve polar analytes. The number of organic compounds (and range of temperature and pressure conditions) for which solubility data is available in the literature is quite small, however, a survey of the solubilities available in a recent review (France *et al.*, 1991) demonstrates that solubility in CO_2 is higher for organics with lower polarities and lower molecular weights than for more polar and larger compounds. As a general rule, organic compounds that can be analyzed by conventional gas chromatography will have sufficient solubility in CO_2 to be extracted, as long as matrix/analyte interactions are not too strong to inhibit the extraction process (i.e., the desorption/kinetic step is too slow). In addition, organics that are quite non-polar but have too high of molecular weights to be considered "GC-able" (e.g., fat triglycerides), can also have high solubilities in pure CO_2.

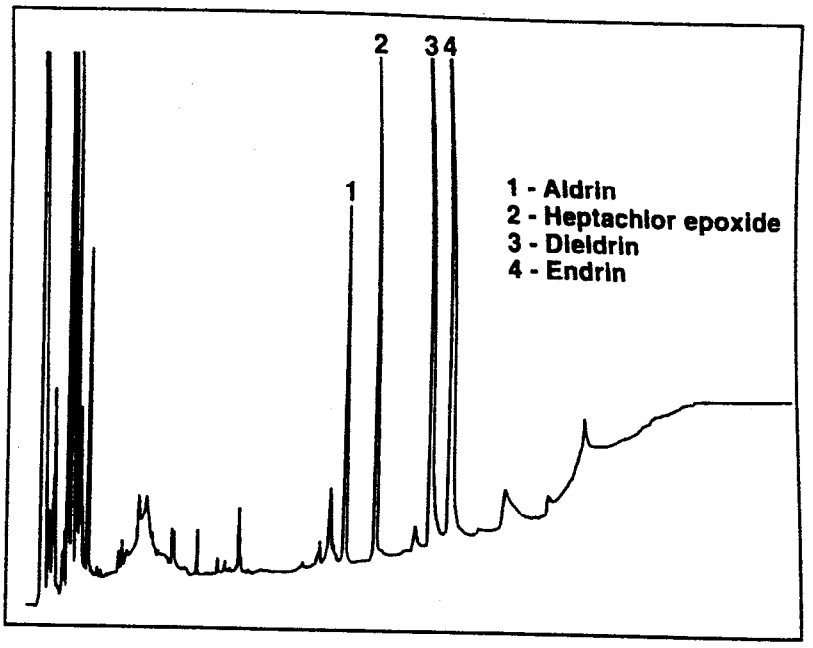

Figure 18 GC/ECD chromatogram of incurred pesticides extracted from chicken fat using fluoroform.

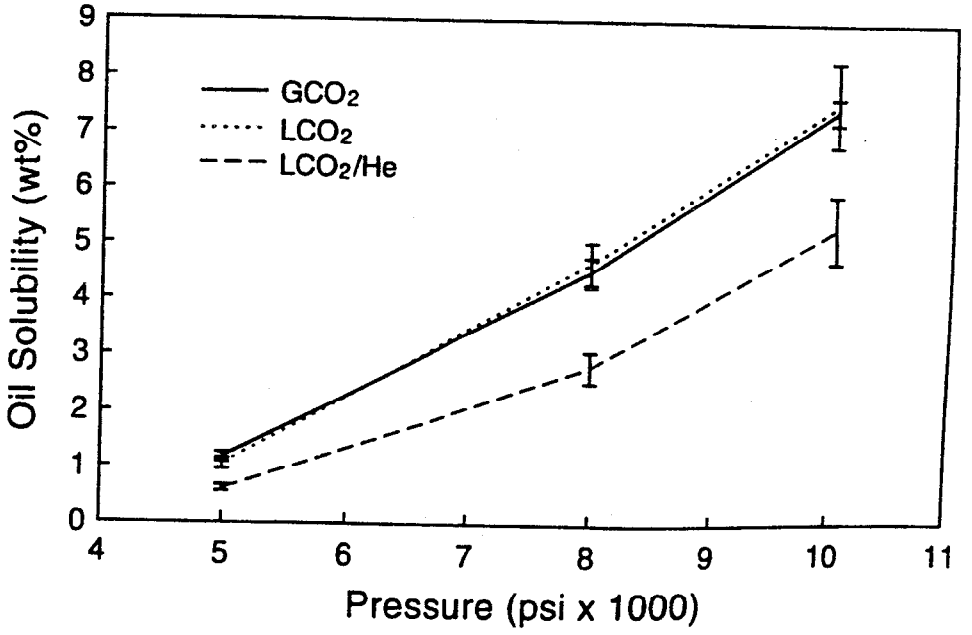

Figure 19 Effect of fluid source on oil solubility as a function of extraction pressure.

There are two general reasons to add a modifier to CO_2 in an effort to increase extraction efficiencies when extraction with pure CO_2 is not efficient. First, modifiers can be added when the solubilities of target analytes are not sufficient to yield reasonable extraction rates, i.e., to increase the extraction rate of samples that are controlled by the solubility/elution process (Step 2). Second, modifiers can be added to interact with the sample matrix in order to enhance the rate of the kinetic/desorption process (Step 1). The selection of modifier, its concentration, and the most effective method for adding the modifier to the extraction system can be guided by a knowledge of whether the need is to increase solubility of the analyte in the bulk fluid (samples controlled by the solubility/elution step) or by a need to increase the rate of the kinetic/desorption step.

Regardless of the reason for choosing a modifier, it is important to determine whether the presence of a modifier causes poorer collection efficiencies (as discussed above), especially for sorbent trapping systems. When possible, modifiers should be selected which do not interfere with subsequent analytical steps (e.g., toluene is a poor modifier choice if UV spectroscopy will be used to determine the analytes). The analyst also needs to recognize that the critical temperature of modified CO_2 will be higher than pure CO_2; therefore, the extraction temperature should be increased to maintain a single phase in the extraction cell. With typical modifier concentrations (e.g., 10 vol% or lower), this can normally be achieved by raising the extraction temperature to 70°C.

VI.B1. Adding modifiers to increase solubility

For extractions limited by the solubility/elution step, the concentration of modifier is typically 5–10 mol% or even greater (vol% is similar to mol% depending on the molecular weight of the modifier compared to that of CO_2), since the need is to increase the solvent strength of the extraction fluid. Based on solvatochromic probes, lower concentrations of modifiers (e.g., 1%) do not change the bulk polarity of CO_2 and, therefore, do not substantially affect the solubility of polar and higher molecular weight analytes (Deye *et al.*, 1990). Therefore, higher concentrations should be used for samples limited by Step 2. Similarly, the constant addition of modifier (e.g., using a dual pump system) should be used so that the solubility enhancement continues throughout the extraction. Selection of modifiers may not be straightforward, since little data exists in the literature for the solubility of organics in mixed supercritical fluids. Valuable information can be gained by surveying the SFC literature for modifiers used with similar analytes. In the absence of relevant literature results, a reasonable starting point is to use a modifier that itself is a good solvent for the target analyte.

As discussed above, modifiers or cosolvents serve two primary purposes in analytical SFE: (1) to increase the analyte solubility in the supercritical fluid, and (2) to overcome matrix interactions that impede SFE. An example that encompasses both these observations is the extraction of relatively polar aflatoxins from a corn matrix. Aflatoxin B1 and its metabolite M1 are only sparingly soluble in SC-CO_2, even at pressures as high as 689 bar, due to their high solubility parameters. Extraction of aflatoxin B1 from a corn matrix is further aided by the addition of cosolvents or binary cosolvent mixtures (Taylor *et al.*, 1996), as will be described in a future section. Although the lipid moiety, cholesterol, is

Table 5 Effect of cosolvents or additives on solubility of solutes in SC-CO_2.

Solute	*Cosolvent/Additive*	*Enhancement Factor*
Acridine	3.5% Methanol	2.3
2-Aminobenzoic Acid	3.5% Methanol	7.2
Cholesterol	9.0% Methanol	100
Hydroquinone	2% Tributyl Phosphate	Greater than 300
Hydroquinone	0.65% AOT*, 6% Octanol	Greater than 200
Tryptophan	0.53% AOT*, 5% Octanol	Greater than 100

*water to surfactant ratio = 10.

soluble in SC-CO_2 to some extent, the results in Table 5 show that its solubility enhancement is significantly improved by the addition of methanol to SC-CO_2. Similarly, other solutes can have their solubilities altered by cosolvents or via the addition of specific agents called additives to the supercritical fluid phase, including surfactants, as shown in Table 5 (Johnston, 1989). We have also found methanol, essential as a cosolvent for the removal of cholesterol from extraction cells containing selective sorbents while practicing the technique of "inverse" SFE.

VI.B2. Adding modifiers to enhance analyte removal from the matrix

When extractions are controlled by the kinetic/desorption step (Step 1), the primary goal of adding a modifier shifts from increasing analyte solubility to an attempt to interact with matrix active sites to enhance the rate of the analyte removal from its binding sites, although a modifier can obviously enhance both Step 1 and Step 2. This situation frequently occurs when the target analytes are reasonably soluble in supercritical CO_2 (e.g., "GC-ables" as discussed above), but are too tightly associated with the matrix to allow efficient extractions (please refer to the discussion on Step 1-controlled samples in Section V.D., above). For such samples, the use of 1% vs. 10% added modifier has been reported to yield similar increases in recoveries, since the major goal of the modifier is to interact with matrix active sites rather than to increase the polarity in the bulk CO_2 (Langenfeld *et al.*, 1994). There appears to be no advantage in extraction efficiencies for 1% over 10% added modifier (Langenfeld *et al.*, 1994), which indicates that methods development can be simplified by simply using 10% concentrations. However, if the modifier interferes with subsequent collection or analysis steps, it may be useful to add modifier at the 1% level for samples controlled by Step 1.

Selection of modifiers for samples controlled by the kinetic/desorption step would be best made on the basis of expected interactions between the target analytes and sample matrix. However, since the nature of such interactions is not known for many samples of interest, it is difficult to select the best modifier without some initial experimentation. Such a modifier survey is quite laborious for dynamic SFE (i.e., constant addition of modified CO_2 to the extraction cell), since it involves changing pump solutions or purchasing different pre-mixed cylinders for each modifier and concentration tested. Fortunately, a much simpler and faster static/dynamic method can be used to survey the effect of several

Table 6 Effect of modifiers on the recovery of essential oil components from savory.

Analyte	*% Recovery: 30 min SFE versus 4 Hours of Hydrodistillation*[a]			
	Pure CO_2	*CO_2/hexane*	*CO_2/acetone*	*CO_2/CH_2Cl_2*
α-pinene	7	38	68	105
p-cymene	42	41	73	114
γ-terpinene	44	41	90	108
carvacrol	63	57	105	119

[a]SFE extractions were performed by direct addition of the modifier to the savory sample followed by 15 minutes of extraction in the static mode, then 15 minutes in the dynamic mode as described in Hawthorne *et al.* (1993a).

modifiers and conditions. The modifier is simply added directly to the sample prior to a static SFE step (e.g., 15 minutes). After the static step is completed, the extracted analytes are recovered from the sample with a dynamic SFE step with pure CO_2. For example, the extraction of essential oils from savory (monoterpenes, sesquiterpenes, and oxygenated monoterpenes) is not efficient with pure CO_2 at 400 atm and 70°C (Hawthorne *et al.*, 1993a), even though the target compounds are generally very soluble at this condition. Savory was then extracted (15 minutes static followed by 15 minutes dynamic) with the addition of 10 vol% of three test modifiers, hexane, acetone, and methylene chloride. As shown in Table 6, the extraction efficiency with hexane modifier was still poor (compared to 4 hours of hydrodistillation), while the extraction with acetone as modifier yielded fairly good recoveries compared to hydrodistillation. However, methylene chloride as modifier yielded the best recoveries of all of the modifiers tested (Table 6).

For environmental samples controlled by Step 1, it has been suggested that the best approach is to determine the relative performance of modifiers with different polarity characteristics (Langenfeld *et al.*, 1994; Yang *et al.*, 1995), e.g., testing a modifier with acid (e.g., methanol), base (e.g., diethylamine), and aromatic character (e.g., toluene). While methanol has been the most frequently-used modifier for environmental samples, modifiers with different polarities are frequently more effective. For example, Yang *et al.* recently compared the extraction efficiencies obtained using methanol, toluene, and diethylamine modifiers for PAHs from marine sediment, diesel soot, and air particulate matter. All extractions were performed using direct addition of the modifier to the sample (15 minutes static followed by 15 minutes dynamic as described above). The enhancement in extraction efficiencies compared to efficiencies achieved with pure CO_2 were generally poor for all samples using methanol modifier, toluene was effective only for the air particulate sample, and diethylamine was effective for all three sample matrices (Yang *et al.*, 1995).

It is important to note that the simple static/dynamic approach with the modifier added directly to the sample is very often sufficient for routine quantitative extractions, in addition to being an excellent way to survey different modifiers and concentrations. This will be true for samples controlled primarily by the kinetic/desorption step (Step 1), as was demonstrated by the results discussed above for the extraction of essential oils from savory and the extraction of PAHs. However, if the solubility/elution step also limits the extraction (Step-2 controlled), adding the modifier directly to the sample using the static/dynamic

procedure will not yield effective extractions since the modifier needs to be supplied throughout the extraction in order to enhance the solubility/elution step. As a practical matter, an analyst extracting samples controlled by Step 1 does not require dual pumping systems (or pre-mixed fluids) to use modifiers, while an analyst extracting samples controlled by Step 2 will require dual pumps (or pre-mixed fluids) if modifiers are needed.

VII. EFFECT OF TEMPERATURE, PRESSURE, AND DENSITY

One of the most frequent errors in developing SFE methods is making the assumption that CO_2 density *alone* controls the solvent strength (and, therefore, analyte solubility). This misperception may be based on the popularity of the correlation proposed by Giddings which relates increased CO_2 density with a higher Hildebrand solubility parameter (i.e., stronger solvent strength) (Giddings *et al.*, 1969). While this correlation is highly useful in providing an understanding of SFE solubilities, it is unfortunately true that many analysts ignore the fact that the correlation is only useful for looking at the effect of pressure on solubilities *at constant temperature*, and the application of Gidding's correlation at constant pressure (by changing density based on temperature changes) is not valid. Because solubility of a particular analyte depends on molecular interactions with the solvent (i.e., solubility is almost always enhanced by higher CO_2 density) *and* by vapor pressure effects (i.e., solubility is enhanced by higher temperature if the analyte has any significant vapor pressure), *both* density and temperature can be used to enhance extraction processes. Therefore, the following sections will discuss general effects of temperature and pressure (which, of course control density) on analyte solubility and extraction behavior.

Note also that, since pressure and temperature define CO_2 density, extraction conditions are correctly described by stating either temperature and pressure, temperature and density, or density and pressure. However, since SFE instruments measure pressure and temperature (instruments reporting density calculate values based on temperature and pressure measurements), it seems most logical to define SFE methods based on temperature and pressure. In addition, since every SFE system is limited by upper pressure and temperature ranges, the following discussion will be based on pressure and temperature.

VII.A. Effect of Pressure and Temperature on Solubility

The concept of "threshold pressure" with respect to supercritical fluids, has it origins in the early studies of "dense gas chromatography" (Giddings *et al.*, 1969), the historical forerunner to SFC. This is defined as the pressure (at a specific temperature) at which the analyte can first be solubilized and detected in the extraction fluid. Therefore, it is dependent on the detection method employed for estimating solubilization and, thus, can vary over magnitudes of concentration (McHugh *et al.*, 1994) depending on whether the detection technique is an element specific GC detector, a TLC spot test, or a gravimetric balance. It will also be dependent on the sample matrix to some extent, hence all of these factors should be specified when quoting a "threshold pressure". Despite these reservations, the threshold pressure is a useful concept since it allows the analyst to know what approximate minimum pressure conditions are required for extracting his analyte from a

Table 7 Effect of pressure on DDT/fat extraction.

Pressure	*Fat Yield (g)*	*DDT Conc. (ppm)*	*% DDT Recovery*
95.2 bar	0	13.7	0
204 bar	2.57	585	75.20
296 bar	13.85	12.9	8.93

Conditions: 20 g of 100 ppm spiked lard, temp. = 60°C, CO_2, flow rate = 4.0 standard liters gas per min.

given sample. Knowledge of this pressure can allow some degree of fractionation to be achieved on samples containing multiple analytes or prevent the SFE of unwanted coextractives from the sample matrix. An example of the latter effect is shown is Table 7 for the SFE of the pesticide, DDT, from fat. Note that a pressure of over 200 bar is required to extract an appreciable quantity of pesticide, while exceeding this pressure (300 bar), dilutes the extract with unwanted fat moieties. Threshold pressures tend to have a weak dependence on temperature and molecular weight. King (King, 1989) has offered a theoretical scheme for predicting threshold pressures for SFE and SFC.

As predicted by the correlation of Giddings *et al.* (1969), increasing pressure at constant temperature (i.e., increasing density at constant temperature) increases the solvent strength of CO_2, and, therefore, increases the solubility of virtually all analytes of interest in SFE. However, it must be remembered that solubility can be enhanced both by increasing CO_2 density and by increasing temperature. At constant pressure, increasing temperature will decrease the density. Therefore, solutes with no significant vapor pressure will show decreased solubility by raising the temperature at constant pressure because of the reduction in CO_2 density. However, solutes with any significant vapor pressure will show higher solubilities with increasing temperature, despite the decrease in CO_2 density that occurs at constant pressure.

These effects are illustrated by the generalized solubility curves in Figure 20 showing the effect of increasing temperature (which, of course, means decreasing CO_2 density) on solubility. Assuming constant pressure, curve "A" would be expected for solutes having significant vapor pressures, i.e., increasing temperature immediately increases solubility despite the concurrent decrease in CO_2 density. Despite limited solubility data in the literature, this case appears to be generally true for "GC-able" compounds. Curve "B" represents compounds with quite low vapor pressures, where the initial decrease in CO_2 density caused by increasing temperature causes lower solubilities. However, as the temperature continues to rise, the vapor pressure effect becomes significant and solubility increases despite the decrease in CO_2 density. For compounds with virtually no vapor pressure at any temperature, the solubility curve may be represented by the left side of curve "B", i.e., the decrease in CO_2 density that occurs with increasing temperature causes decreasing solubility over all reasonable temperature and pressures.

Curves "A" and "B" in Figure 20 can also be used to represent the solubility of a single compound at two different pressures (the pressure for curve A being higher than the pressure for curve B). Thus, at lower pressures the initial increase in temperature causes a decrease in solubility because the CO_2 density is lowered too much. However, at the same pressure but a higher pressure (curve A), the CO_2 density is sufficient to allow the enhancement from the increased solute vapor pressure to be realized.

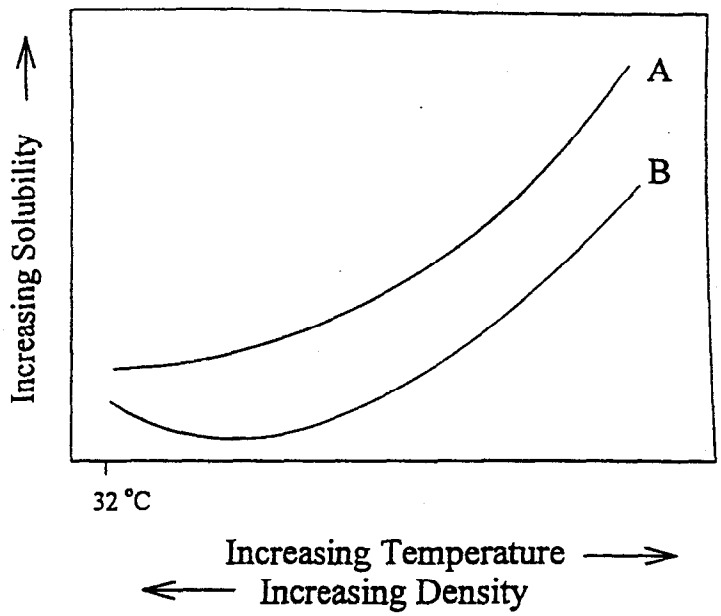

Figure 20 Generalized curves showing possible effects of temperature and density on solubilities in CO_2. Refer to the text for discussion of curves A and B.

Evaluating the effect of temperature on solubility is hampered by the general lack of solubility data at temperatures above 80°C. However, recent studies have extended the range of solubility data for several organics up to 250°C (Miller *et al.*, 1995, 1996). Figure 21 shows typical results obtained for organic solutes which show behavior like curve "A" in Figure 20, i.e., have enough vapor pressure to be "GC-able". As shown by the solubility data for benzo[*ghi*]perylene in Figure 21, the solubility is increased much more by raising the temperature 40 to 150°C (the upper limit of commercial SFE instruments) than by raising the pressure (at 40°C) from 100 to 450 atm, despite the decrease in CO_2 density which occurs with the temperature increases. It is interesting to note that even a fairly non-volatile compound like benzo[*ghi*]perylene (melting point = 278°C, boiling point = 500°C) shows such a large enhancement in solubility by simply increasing the temperature from 40 to 150°C. Recent solubility studies on organic solutes including several polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and pesticides at temperatures as high as 250°C have demonstrated very high increases in solubilities at pressures normally used for SFE (e.g., 150 to 400 atm) (Miller *et al.*, 1996, 1996a). It must be noted that the use of higher temperatures may lead to thermal degradation of some analytes. An additional limitation is that most commercial instruments are limited to 150°C. However, as shown in Figure 21, 150°C is sufficiently high to yield very large increases in solubilities.

Tripalmitin is used in Figure 21 to demonstrate the effect of temperature and pressure on the solubility of a compound showing behavior similar to curve "B" in Figure 20. At

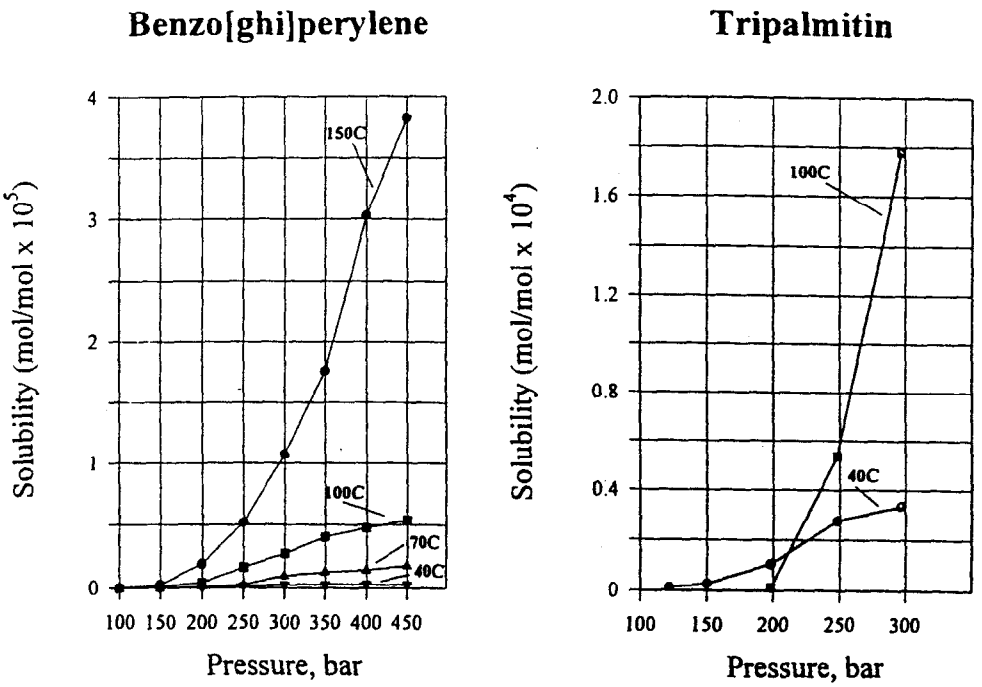

Figure 21 Effects of temperature and pressure on the solubilities of the PAH benzo[*ghi*]perylene and the triglyceride tripalmitin in supercritical CO_2. Results are adapted from Miller *et al.* (1995, 1996).

However, as shown in Figure 21, 150°C is sufficiently high to yield very large increases in solubilities.

Tripalmitin is used in Figure 21 to demonstrate the effect of temperature and pressure on the solubility of a compound showing behavior similar to curve "B" in Figure 20. At lower pressures (e.g., 193 bar), the solubility of tripalmitin drops when the temperature is raised from 40 to 100°C, because the CO_2 density at 100°C is simply not sufficient to allow significant solvation. However, as the pressure is increased from 198 to 248 bar, the CO_2 density increases enough that the effect of temperature begins to result in higher tripalmitin solubilities. As the pressure is further increased to 297 bar, the solubility of tripalmitin is considerably higher at 100°C than at 40°C, despite the fact that the CO_2 density decreases from 0.92 g/ml to 0.66 g/ml at 40 and 100°C, respectively.

Practical effect of pressure and temperature on SFE recoveries

For samples controlled by the solubility/elution step (Step 2) raising the extraction pressure (at a given temperature) will generally increase extraction efficiencies since the solubility of the analyte will increase (although pressures above ~400 bar generally have little effect). Similarly, if raising the extraction temperature increases analyte solubility (as discussed above), raising temperature will be effective for increasing the extraction rate for samples

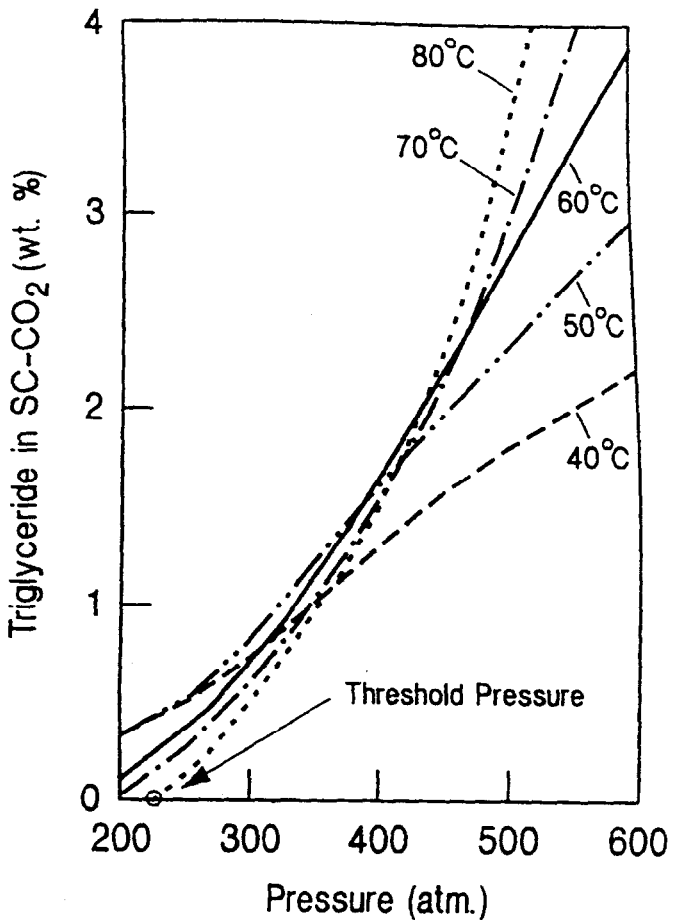

Figure 22 Solubility of soybean oil triglycerides in SC-CO_2 as a function of temperature and pressure.

The solubility trend with temperature for the model triglyceride, tripalmitin, has a considerable effect on the extraction of fatty materials from food and other related by SFE. Figure 22 shows the inverse temperature dependence for soybean oil triglycerides as a function of pressure during SC-CO_2 extraction. Again, at lower pressures we see that an increase in temperature reduces the solubility of the oil in the fluid phase. However, at higher pressures, oil solubility is dramatically increased with a rise in temperature, suggesting that the optimum conditions for rapid determination of fat or oil in a matrix are at 689–827 bar and temperatures in the range of 80–100°C (King, 1993). This effect can be rationalized by realizing that the relative respective cohesive energy densities of the analyte (solute) in the SC-CO_2 (solvent), and their change with temperature are what governs solubility enhancement. For example, the change in the solubility parameter (the square root of the cohesive energy density) is less for a triglyceride, such as tristearin, than for SC-CO_2, as a function of temperature. Although the solubility parameter of both

solute and solvent in this case decrease with temperature, they are closer in numerical value at the higher, rather then the lower temperature. Hence, greater solubility of the solute in the solvent is to be anticipated at the higher temperature since their solubility parameters are closer. Similar results have been reported by King *et al.* (1993) for the extraction of multi-pesticide residue species from a grain matrix. Here, extraction at 345 bar and 60°C gave the best overall average recoveries for eight individual pesticides as opposed to SFE at 172 bar and 60°C.

For samples that are controlled by the kinetic/desorption step (Step 1), raising the extraction pressure is often not effective since the extraction is not limited primarily by analyte solubility, although it should also be noted that raising pressure can still be tried since it is highly unlikely that any reduction in extraction rates will occur. For such samples, the rate of extraction is often enhanced by raising the extraction temperature (Hawthorne *et al.*, 1994; Langenfeld *et al.*, 1993, 1995; Robertson *et al.*, 1994). Unfortunately, it is difficult to demonstrate whether the increased extraction rates obtained by raising the temperature are a result of increased solubility (i.e., for "GC-able" solutes), or are a result of an increased rate of the kinetic/desorption step. However, since raising temperature increases the rates of many chemical reactions, it seems reasonable that the enhancement often seen by increasing extraction temperature is a result of faster desorption kinetics during Step 1, as been proposed by Pawliszyn *et al.* (Langenfeld *et al.*, 1995; Pawliszyn, 1993). In any case, increasing temperature has been found to be very effective strategy for increasing extraction efficiencies for "GC-able" analytes that are tightly bound to the sample matrix.

Recent reports have demonstrated the ability of elevated temperatures to yield higher extraction efficiencies, particularly for environmental pollutants (Hawthorne *et al.*, 1994; Langenfeld *et al.*, 1993, 1995; Robertson *et al.*, 1994). For example, the recoveries of several different classes of organic pollutants using 30- to 40-minute extractions with pure CO_2 at 50°C (CO_2 density of 0.9 g/ml) and 200°C (CO_2 density of 0.5 g/ml) have been compared to concentrations of the same analytes based on 18 hours of Soxhlet extraction (Hawthorne *et al.*, 1994; Langenfeld *et al.*, 1993). As shown in Table 8, the recoveries achieved with the higher density condition (40°C), were generally much lower than the recoveries achieved at the lower density (200°C) condition. Note also that extraction with pure CO_2 at 200°C generally gave excellent recoveries compared to the Soxhlet extractions. For particularly difficult extractions, it has also been reported that the combination of high temperature and the addition of a modifier increases extraction efficiencies (Yang *et al.*, 1995).

It is important to note three potentially-important limitations to the use of higher temperatures for analytical SFE. First, the analyst must be sure that no thermal degradation of the target analytes occur (which can be determined with a spike recovery study). While thermal degradation is highly unlikely for many environmental pollutants (e.g., PAHs and PCBs) because of their thermal stability, it would seem much more likely to be a problem with biological samples and related analytes. Second, the results thus far have been conducted on "GC-able" analytes. As discussed above, analytes with very low vapor pressures may not show large enhancements in solubility. Third, most commercial instruments are limited to 150°C, while the early reports demonstrating higher extraction rates with higher temperatures have used temperatures up to 200°C. However, recent

Table 8 Effect of SFE temperature on semi-volatile pollutant recoveries from soil using pure CO_2.

Analyte	*Matrix*	*Soxhlet conc. µg/g (%RSD)*	*% Recovery SFE vs. Soxhlet (%RSD)*[a]	
			50°C	*200°C*
PAHs	air particulates			
fluoranthene		7.1 (7)	49 (9)	142 (10)
benz[*a*]anthracene		2.6 (12)	46 (6)	102 (6)
benzo[*a*]pyrene		2.9 (17)	23 (20)	70 (8)
PCBs	sediment			
2,4,4'-trichloro-		2.21 (5)	38 (2)	90 (2)
2,2',3,5'-tetrachloro-		1.07 (11)	64 (1)	108 (2)
2,3',4,4'5-pentachloro-		0.51 (2)	75 (2)	124 (3)
2,2'3,3'4,4'5-heptachloro-		0.11 (9)	96 (1)	144 (3)
Pesticides	soil			
atrazine		0.47 (21)	72 (2)	111 (19)
prometon		0.69 (24)	74 (5)	119 (24)
Heterocyclics	soil			
benzothiazole		103 (41)	21 (20)	107 (33)
diphenylamine		188 (40)	13 (11)	143 (15)
thiocyanodiphenylamine		877 (29)	4 (35)	77 (28)
acridine		118 (52)	6 (21)	87 (26)
Chlorophenols	soil			
2,6-dichlorophenol		4.8 (41)	14 (40)	103 (21)
2,4,5-trichlorophenol		25 (12)	33 (33)	102 (11)
pentachlorophenol		153 (38)	1 (150)	114 (18)

[a]SFE was performed in triplicate for each sample at 405 bar for 30 minutes (for the pesticides, heterocyclics and chlorophenols) or 354 bar for 40 minutes (fair particulates and sediment). Soxhlet extractions were performed for at 18 hours in triplicate (for the pesticides, heterocyclics, and chlorophenols) or were used for the certification of the NIST standard reference materials (air particulates and sediment). Results are adapted from references 50 and 90.

solubility studies (Bartle *et al.*, 1991; Miller *et al.*, 1996, 1996a) and extraction studies Bøwadt *et al.* (1995) have demonstrated that large enhancements in solubilities and extraction efficiencies can be achieved at 150°C, as well as at higher temperatures.

VIII. SELECTIVITY IN SFE

VIII.A. Selectivity versus Bulk Matrix Organics from Soils and Sediments

One of the important reasons to perform SFE is that the extracts are frequently much cleaner (contain lower levels of extracted matrix material) than organic-solvent based extractions (e.g., Soxhlet extraction, sonication, accelerated solvent extraction). For example, SFE extracts of semi-volatile pollutants (e.g., PAHs, PCBs) from contaminated

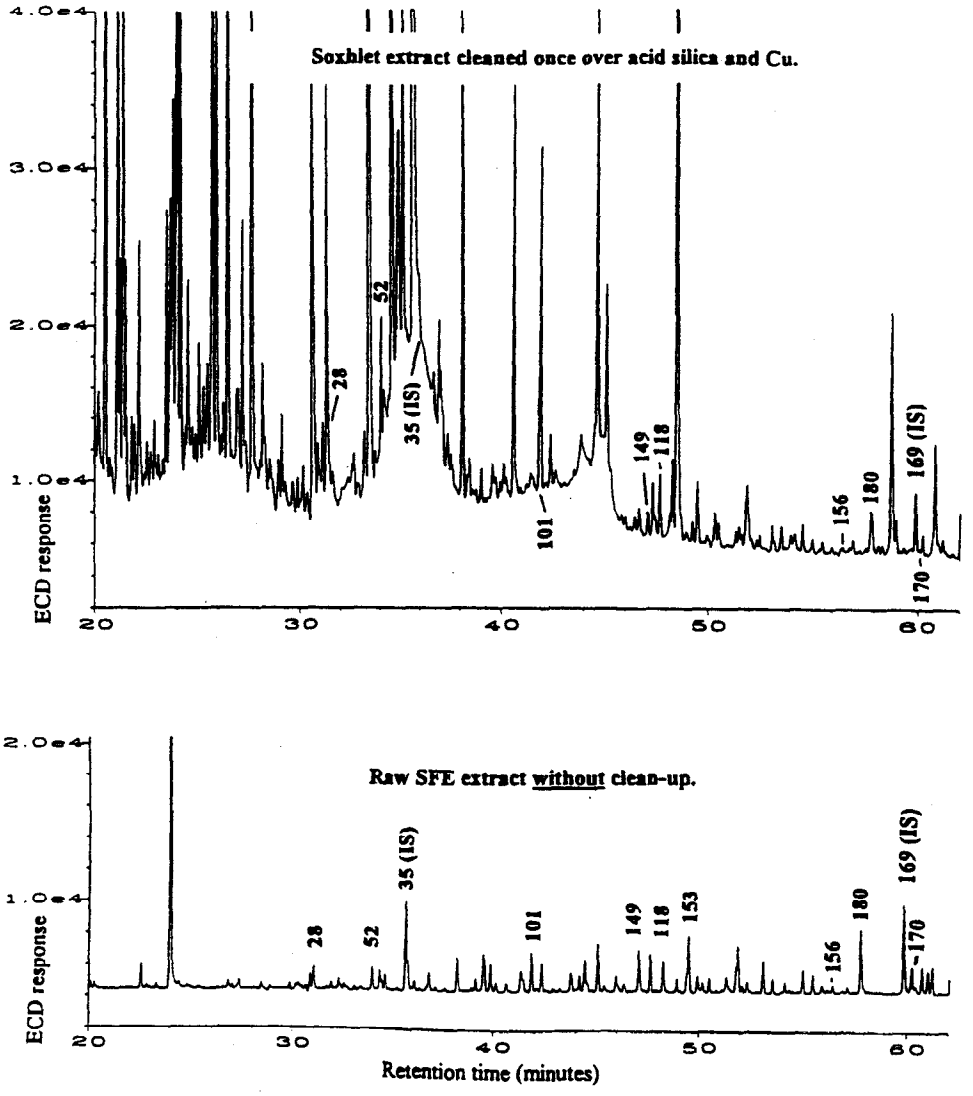

Figure 23 Comparison of GC/ECD chromatograms of a Soxhlet extract of marine sediment (NIST SRM 1941a) after clean-up over acid silica and copper adsorption (top) and a raw SFE extract (using copper in the SFE cell to adsorb elemental sulfur), bottom. The numbers on the chromatograms indicate the retention time of individual PCB congeners. Chromatogram courtesy of Søren Bøwadt.

One of the important reasons to perform SFE is that the extracts are frequently much cleaner (contain lower levels of extracted matrix material) than organic-solvent based extractions (e.g., Soxhlet extraction, sonication, accelerated solvent extraction). For example, SFE extracts of semi-volatile pollutants (e.g., PAHs, PCBs) from contaminated soils are typically clear to light yellow, while the Soxhlet extract of the same samples are nearly black. Often the SFE extracts can be analyzed immediately without the need for

Table 9 Sorbents used for fractionation in analytical SFE.

aluminas	silica gel
silicas	Florisil
celite	hydromatrix
dessicant materials	modified silicas
adsorbent disks	synthetic resins/foams

any post-extraction clean-up steps. However, the Soxhlet extract of the same sample shows massive interferences even after the extract is subjected to a clean up step on acid silica and adsorption of the elemental sulfur on copper. In fact, the Soxhlet extract required three clean-up steps by acid silica fractionation before the ECD detector background was low enough that the PCBs could be determined (Bøwadt).

It should be noted that the addition of modifiers in SFE to increase extraction efficiencies does carry a penalty in generating extracts with more extracted matrix material (as indicated by darker extracts than those obtained using pure CO_2). Interestingly, using higher temperatures with pure CO_2 to increase extraction efficiencies of "GC-able" organics (as discussed above) typically does not yield nearly as much co-extracted material in the extract as either organic solvent extractions or as using modifiers in CO_2, presumably because the use of higher temperatures with pure CO_2 does not cause increases in the solubility of matrix components (e.g., polar and high molecular weight materials such as humic acids).

VIII.B. Selectivity in Biological Samples

Many examples of optimizing selectivity in SFE have already been alluded to in the previous sections, but some mention of the special problems posed by biological and natural product matrices during SFE should be mentioned in this section. Although it is a mute argument as to what sample matrices are the most difficult to extract specific analytes from via SFE, it is probably fair to say that the molecular complexity of many natural and biological samples poses specific problems to SFE with respect to extraction specificity. Whereas the previously mentioned environmental matrices are simplified somewhat due to their high content of inorganic matter, the level of extractables in food and biological matrices is quite variable, although it is generally agreed that carbohydrate and protein matter have limited solubility in SC-CO_2 under typical SFE conditions. It is the high propensity of SC-CO_2 to extract lipid matter from natural products however, that causes much of the selectivity problem in the SFE of these materials. This has lead to the integration of cleanup techniques into the SFE schemes.

Table 9 tabulates typical sorbents and materials that have found use in SFE. The sorbents listed generally tend to be "normal" phase column packing materials according to HPLC classification systems (Snyder, 1971). This is not coincidental, since the elutropic strength of SC-CO_2 even at high pressures, is limited to emulating non-polar to medium polarity liquid eluents. Aluminas, silicas, bonded or modified silicas, diatomaceous earths, and Florisil have all been cited in the SFE literature. These can be added directly to the extraction cell as a segregated bed from the sample matrix or have occasionally been successfully mixed directly with the sample matrix to effect a fractionation during the SFE.

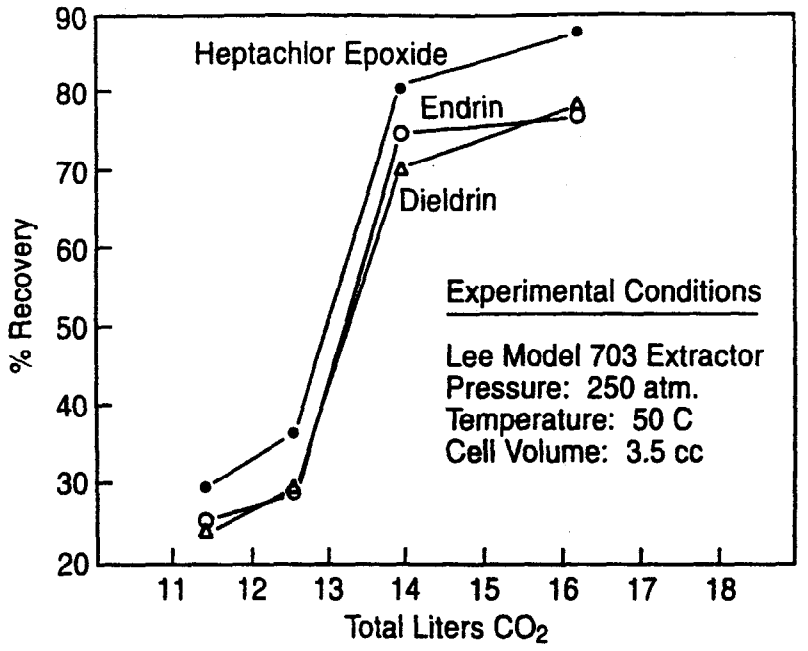

Figure 24 Percent (%) pesticide recovery through extraction cell loaded with alumina for sample cleanup.

An example of the isolation of pesticides from lipid matter during the SFE of poultry fat is shown in Figure 24. Here, neutral alumina, heat activated, whose activity level has been adjusted by the addition of water (France *et al.*, 1991a), is used to retain interfering lipid moieties while the pesticides; endrin, dieldrin, and heptachlor epoxide, are eluted with high recovery. It is important to know when the pesticides breakthrough so as to adequately time the collection of fractions devoid of fat. This described approach has proven popular in many applications of SFE, including the selective isolation of PAHs and PCBs from environmental matrices (Bøwadt *et al.*, 1995). Care must be taken, however, to realize that these crude "chromatographic columns" are governed by the same elution principles that occur in other forms of chromatography. For example, the cleanup of a spiked lard sample on silica can be negated if the SFE is run excessively, thereby promoting breakthrough of the interfering compounds from the sorbent bed. This is illustrated in Figure 25 where small quantities of methanol have been added to aid in the recovery of the listed pesticides. Coextracted lipids remain acceptably low up to a 2% methanol addition level in the SC-CO_2, however, a 3% methanol level proved excessive and lead to the elution of interfering lipid compounds in the desired extract (Field *et al.*, 1992).

IX. ANALYTICAL REACTIONS IN SFE

Analytical reactions during SFE provide the analyst with another approach to improve extraction selectivity, detection of extracted analytes via derivatization, increases in analyte

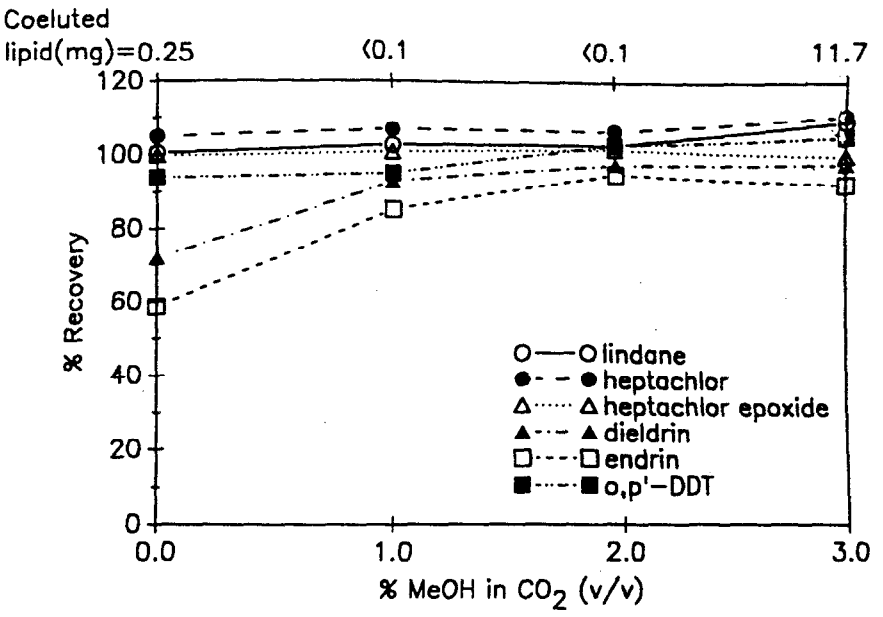

Figure 25 Effect of methanol modifier on cleanup of spiked lard during SFE.

volatility, and enhanced solubility of the target analyte(s) in the extraction fluid. This can encompass quite a wide range of analyte types, ranging from lipids and pesticides, to inorganic species, as shown in Table 10. Many of the cited reactions utilize well known derivatizing agents that have been used in GC and HPLC methodology or as a reactant designed to improve the solubility of a normally insoluble analyte in SC-CO_2. The latter is best illustrated by the use of fluorinated "designer" ligands for enhancing the solubility of metal species in SC-CO_2 (Laintz *et al.*, 1991). One must be cautious when using derivatizing agents as reactants in SFE, particularly with matrices that are complex, since the extract may turn out to have more extracted matrix species than obtained through a conventional SFE approach.

Table 10 Derivatizations applied in analytical SFE.

Alkylating Agents/BF3 — Acidic Herbicides
Ion-Pairing Agents/TMPA — Ionic Surfactants
Pentafluorobenzyl Esters/TEA — Phenols, etc.
Silylation Reagents — Matrix Derivatization
5% HCl/Methanol/XAD-4 Resin — Fatty Acids
Trimethylphenylammonium Hydroxide — Fatty Acids
Acetic Anhydride/AG-1-X8 Resin — Phenols
Esterification — Tocopherols
Lipase/Alcohol Transesterification — Fatty Acids
Alumina/Alcohol — Fatty Acids
Ligand Reactions — Assorted Metals/Inorganics

Several examples illustrating the breadth of this approach are described below. The use of hydrophobic ion-pairing agents have proven facile for characterizing surfactants in sewage sludges (Field *et al.*, 1992), and a similar approach has worked well for difficult-to-extract acidic herbicides, although the mechanism of extraction/reaction remains controversial (Lopez-Avila *et al.*, 1993). Silyation reagents have been applied to environmental matrices and can be used to extract sugars as illustrated by the SFC studies of Chester *et al.* (1986). Fatty acid derivatization is critical to many areas of scientific endeavor and a number of novel approaches have been developed that can be coupled with SFE. Trimethylphenylammonium hydroxide has been used to derivatize fatty acids by White and coworkers (White *et al.*, 1991) and transesterification over XAD-4 resin via the addition of 5% HCl/methanol has proven efficacious for the production of methyl esters. More recently, King and coworkers (Snyder *et al.*, 1996, 1996a) have shown the utility of lipases in the derivatization of FAMES via transesterification. Both off-line and on-line modes of SFE/SFR (supercritical fluid reaction) have been employed in these lipase catalyzed transesterification, and the technique can be employed on small samples. Caution must be exerted to not exceed the upper pressure and temperature limits tolerated by the lipase (approx. 276 bar and 60°C) during the SFE/SFR. Water can also be a mitigating factor in successful lipase-initiated derivatizations, and matrices having high water content should probably be freeze dried or purged with SC-CO_2 to remove excessive water (Snyder *et al.*, 1996). The integration of a reaction sequence with the SFE step can take on quite a high degree of sophistication as shown in Figure 26. Here a totally automated on-line

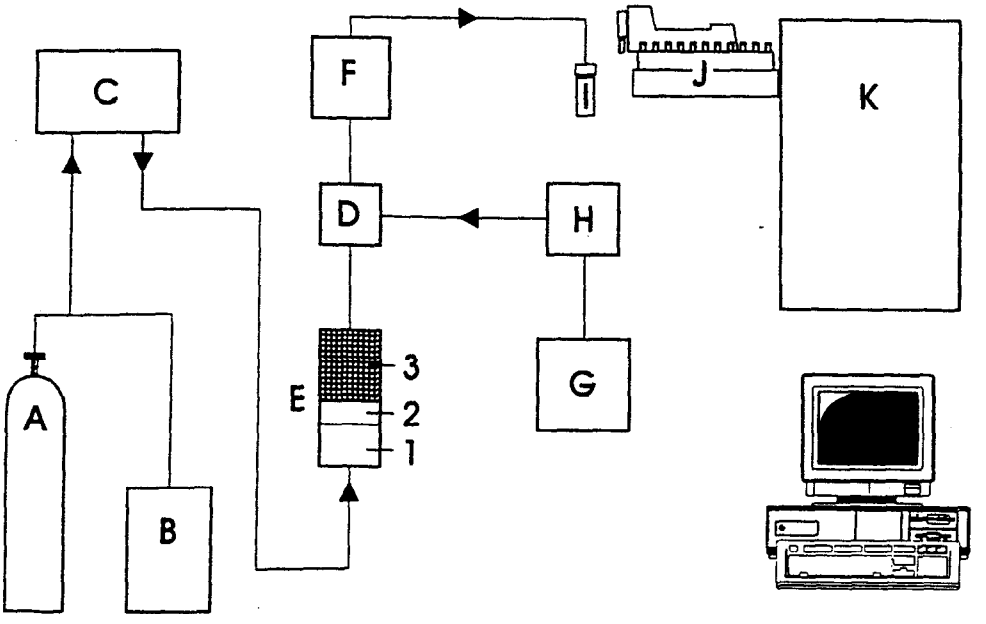

Figure 26 Automated SFE/SFR/GC analyzer for the determination of fat content in foods.
Legend: (A) cylinder; (B) methanol; (C) high pressure pump; (D) valve; (E) extraction cell — (1) sample, (2) glass wool plug, (3) supported lipase; (F) analyte trap; (G) hexane rinse solvent; (H) rinse solvent pump; (I) sample vial; (J) GC auto injector tray; (K) gas chromatograph.

Table 11 Day-to-day variables for SFE and liquid solvent extraction.

Supercritical	*Liquid*
Solvent choice (limited)	Solvent choice
Time	Time
Sample size (and cell size)	Sample size
Pressure	Apparatus (e.g., Soxhlet, sonication)
Temperature	
Flow rate and mode (static/dynamic)	
Modifier choice and concentration	
Collection device (and conditions)	

FAME synthesizer is shown in which the alcoholic reagent is blended with the SC-CO_2 before the extraction cell and then passed sequentially over the sample to be extracted followed by esterification of the extract via the lipase also contained in the extraction cell. The derivatized sample is then captured on a sorbent contained in the analyte collection trap, rinsed into a vial with a small aliquot of solvent, which is robotically transferred to a gas chromatographic auto-injector for analysis. A similar derivatization approach can be used to extract metallic species in which a SC-CO_2-compatible ligand is initially solubilized, then passed through the extraction cell, thereby selectively extracting the metallic species of interest. This is a relatively new area of research and promises an extension of SFE for inorganic analysis (Lin *et al.*, 1994).

X. GENERALIZED APPROACHES TO SFE

X.A. Variables in the SFE Experiment

One reason for the confusion surrounding SFE methods development is the fact that the SFE experiment has more degrees of freedom than can actually be used during the experiment than an organic solvent extraction. This allows extractions to be performed with more selectivity (as discussed above), but does require more understanding of the relevant parameters. Fortunately, a general awareness of the importance and effects of these parameters can greatly reduce the confusion of an analyst new to SFE. While SFE and organic solvent extractions are governed by the same physicochemical principles and influenced by nearly the same variables, more day-to-day variables are controlled by the analyst doing SFE as outlined in Table 11. The most important differences between conventional solvent extraction and SFE is the ability to change pressure and temperature to control the solvent strength of the fluid, as well as the ability to mix fluids (add modifiers) with a much greater range of concentrations and identities than can be used in liquid solvent extraction. One should also be aware of changes in sample morphology during SFE, i.e., pressure may expand or compress some sample matrices making them easier or more difficult to extract. An excellent example of this is during the SFE of polymers, where the application of pressure may cause swelling of the polymer, as well as affect appreciably such parameters as the glass transition temperature (Tsirule *et al.*,

Table 12 Different approaches to obtain high extraction efficiencies of PCBs from a "worst case" sediment (SRM 1939).

Fluid	CO_2	CO_2	CO_2	CO_2	CO_2	CO_2	$CHClF_2$	H_2O
Modifier (v/v)	5% MeOH	10% MeOH	10% aniline	–	–	–	–	–
Pressure (bar)	405	405	405	660	152	378	405	50
Temperature (°C)	70	80	80	200	200	97	100	250
Static time (min)	0	5	5	0	0	10	0	0
Dynamic time (min)	40	10	10	40	40	60	40	15
Dynamic flow rate (ml/min)	0.7	1.0	1.0	0.8	0.8	1.0	0.7	1.0
Reference	73	64	64	90	90	92	73	103

1994). However, these additional degrees of freedom are often more frustrating than useful to an analyst new to SFE. Therefore, comments on optimizing SFE conditions and some general "rules of thumb" are given below.

X.B. What's Needed to Optimize SFE Conditions

One of the most unfortunate myths about SFE is that all of the variables discussed above must be "optimized" for an extraction to yield reasonable quantitative results. This is certainly not the truth, since many combinations of SFE conditions can yield good extraction efficiencies. For example, the PCBs on NIST river sediment (SRM 1939) are much more difficult to extract than most PCB-contaminated soils and sediments, and will not be efficiently extracted at "normal" SFE conditions (e.g., 300 to 400 bar, 40 to 60°C). However, quantitative extractions of PCBs (~75 to 130% recoveries compared to the values reported by NIST based on two sequential 16-hour Soxhlet extractions) from this sample have been achieved with extraction times as short as 15 minutes using several different SFE conditions as shown in Table 12. Various conditions that have been successful include using a variety of different modifiers under dynamic and static flow, by using alternate supercritical fluids such as $CHClF_2$, using pure CO_2 at higher temperatures, and even with the use of subcritical water (Yang *et al.*, 1995b). Note also that the "stronger" conditions used to achieve high recoveries from the difficult NIST river sediment work perfectly well for all PCB-contaminated soils and sediments (based on the author's and co-workers experience with ~30 different real-world samples).

It is also important to note that a single SFE condition often will extract a large range of analyte types. For example, the use of pure CO_2 at 150°C for 30 minutes gave very good recoveries (typically 80 to 120% versus 18-hour Soxhlet extractions) for PCBs and PAHs from a variety of soils and sediments. As shown in Table 8, the use of pure CO_2 at high temperatures gave good recoveries of a wide range of semi-volatile pollutants, and it is reasonable to expect that nearly all of the compounds on the U.S. Environmental Protection Agency's list of semi-volatile organic pollutants would be efficiently extracted with pure CO_2 at 150°C.

Similar considerations can also apply when extracting food or biological samples, although there are some subtle differences to consider. Extraction pressures to 689 bar should generally be available, since removal of analytes from biological matrices can

involve a diversity of sample types and analytes. Total removal of water, always present in many types of biological samples and food products, is often not necessary since low sample water content (below 10 wt.%) can usually be controlled by selection of extraction conditions or addition of desiccants into the extraction scheme.

X.C. Selecting Conditions for SFE

The reader should first review the previous sections on collection efficiencies, sample homogeneity, and decide upon the definition of "quantitative" recovery to be used. The next task is to select initial conditions and extract representative samples to determine extraction efficiencies. The following sections give advice on selecting these extraction conditions based ~20 years of accumulated experience developing analytical SFE methods of the two co-authors. Before starting the discussion, it is important to note that different investigators may suggest different approaches, just as several different SFE conditions have been developed that yield quantitative recoveries of PCBs as described above (Table 12). The advice given below is, essentially, the approach that the authors of this chapter would follow in their own laboratories, and will hopefully be useful to others. The discussions below are divided by sample matrices rather than by analytes because different approaches to SFE conditions are controlled as often by the sample matrix as by the analytes (not to mention that the two co-authors' experiences are divided by sample matrices). For example, extracting PCBs from sediment is generally limited by the desorption/kinetic step (Step 1) because of the strong interactions of the analytes with the matrix that has resulted from environmental aging. The SFE conditions must, therefore, overcome the analyte/matrix interactions in order that Step 1 is reasonably fast (e.g., by raising temperature or adding modifier). In contrast, extracting PCBs from biological tissue is much more likely to be controlled by the solubility/elution step (Step 2), since the PCBs will be associated with the fat in the sample, and their extraction will be enhanced by SFE conditions that enhance Step 2 (e.g., faster flow rates, higher pressures).

X.C.1. SFE conditions for environmental solids

Environmental samples vary greatly in their matrix characteristics, and in more cases are controlled by the kinetic/desorption step (Step 1) than by the solubility/elution step (Step 2), with the notable exception of more polar and higher molecular weight organics which often are controlled by Step 2 because of their lack of solubility in CO_2. The analyst should choose representative real (not spiked) samples for methods development, and if possible, the analyst should choose a sample known to be difficult to extract (e.g., the NIST SRM 1939 for PCBs as discussed above) since SFE conditions that yield high recoveries for the difficult samples will also work for samples which have weaker analyte/matrix interactions. Although not always true, samples with lower contamination levels and longer aging times generally will be more difficult to extract, since the pollutants will be more tightly associated with active sites on the matrix, and are, therefore, better choices for methods development.

Several general rules can be given for SFE of various classes of organic pollutants from environmental solids such as soils, sediments, sludges, and air particulate matter (i.e., non-

biological samples). The conditions suggested below (based on the author's experience) will frequently yield satisfactory recoveries for a broad range of organic pollutants and no further development will be needed. As stated above, several different SFE conditions may be equally useful, but some have practical disadvantages and will not be suggested (e.g., supercritical $CHClF_2$ is an excellent extraction fluid, but the use of Freons is not as acceptable as CO_2). In addition, the suggested approaches will focus on methods that are simplest (and least expensive) to perform using commercial instrumentation.

Volatile Organic Compounds (VOCs)

VOCs in environmental samples include organic compounds with relatively high vapor pressures and are frequently a result of contamination from commercial solvents and fuels such as gasoline. For the more volatile VOCs, purge and trap analysis is less expensive and more reliable than SFE, and should be considered. However, there is often a desire to determine a range of organics from a single extraction. For example, purge and trap is applicable to only the more volatile end of gasoline components (e.g., benzene), but can fail to yield high recoveries of less volatile compounds which are also present in gasoline (e.g., higher molecular weight alkyl benzenes). If properly performed, SFE has the advantage that both VOCs and semi-volatile organic pollutants can be determined from a single extraction method. For example, extraction and quantitation of gasoline-to-diesel range organics requires a purge and trap and an organic solvent extraction by conventional methods, but SFE has been used to determine the entire range of hydrocarbons with a single extraction (Yang *et al.*, 1995a).

Organic pollutants that have enough vapor pressure to be considered VOCs have high solubilities in CO_2 and are weakly associated with the sample matrix. Therefore, mild SFE conditions (e.g., 350 bar or less, 40°C, 20 to 30 minutes) with pure CO_2 will efficiently extract VOCs, and virtually no methods development is needed on the extraction step. However, the collection efficiencies of extracted VOCs can be very difficult, particularly for the more volatile compounds. The reader interested in using SFE for VOCs should carefully review the collection section (above) as well as the relevant references.

Semi-volatile organics

For the purpose of this discussion, semi-volatile organics are compounds with lower vapor pressures than VOCs, but have sufficient vapor pressures to be "GC-able" by conventional capillary gas chromatography. These compounds have received the most attention in the SFE environmental literature because they typically have sufficient solubility in supercritical CO_2 to be extracted, and because of the desire to replace the conventional organic solvent extraction methods. For the more volatile semi-volatiles (e.g., naphthalene), the analyst must be quite careful with collection efficiencies; however, the major problem with semi-volatiles is the extraction step.

For most semi-volatiles, extraction with pure CO_2 at "typical" SFE conditions (e.g., 350 to 400 bar, 40 to 50°C, 30 minutes, CO_2 flow rate of 1 to 2 m/minute), will extract loosely-bound analytes (e.g., spiked analytes or incurred analytes that are not tightly associated with matrix active sites), but unfortunately, can often fail to yield high recoveries of

pollutants that have been aged in the matrix in the environment (see the discussion on determining quantitative extractions, above). This is, essentially, because the extraction of loosely bound analytes (whether spiked or not) is controlled by the solubility/elution step (Step 2), while the extraction of more tightly bound analytes is controlled by the desorption/kinetic step (Step 1).

If recoveries of semi-volatile organics from real samples are not satisfactory with the "typical" SFE conditions given above, the next simplest step is to raise the temperature to the upper limit of the instrument (typically 150°C for most manufacturers) and repeat the extraction using the same SFE conditions as for the lower temperature extraction. Raising the temperature should increase analyte solubility (thus enhance Step 2), as well as increase the rate of Step 1 as discussed above. While raising SFE temperatures has not yet gained popularity, we have found it the single most effective approach to increasing recoveries of semi-volatile organics from environmental samples. In addition, the use of high temperature with pure CO_2 frequently increases extraction efficiencies at least as well as adding modifiers, yet eliminates the problems in collection efficiencies and occasional need to remove the modifier prior to analysis (as discussed above), as well as being simpler and less expensive. It should be noted that raising the extraction pressure to the upper limit of the instrument will certainly not hurt extraction efficiencies, but raising pressures above ~400 bar have generally not been shown to increase efficiencies since CO_2 density does not increase substantially at higher pressures.

As analytes get more polar (e.g., chloroanilines, nitrotoluenes), it becomes more likely that solubility will be a problem and an added modifier will be needed. However, adding modifier also increases the quantity of more polar matrix material that is extracted, while raising temperature yields a much cleaner extract. Should raising the temperature with pure CO_2 fail to give adequate recoveries, the addition of modifiers should be investigated using the static/dynamic approach described above. (Please refer to the discussion on modifiers concerning selecting modifiers and surveying their performance, as well as the cautions on using modifiers with sorbent trapping systems.) A typical survey could utilize methanol, toluene, and diethylamine to represent acid, aromatic, and base character, respectively. Extractions should use the same conditions as above, with the exception that a short (e.g., 15 minute) static extraction should precede the dynamic extraction to allow the modifier (which is added directly to the sample) to interact with the sample before being eluted from the SFE cell. Temperatures for extraction are typically raised just enough to ensure a single-phase system (e.g., 70–80°C for a 10 vol% modifier addition). However, Yang *et al.* have recently reported that combining modifiers and raising the SFE temperature is even more effective for particularly difficult samples (Yang *et al.*, 1995a).

Non-volatile and polar organics

In contrast to most semi-volatile organics, organics that are too non-volatile or too polar to be analyzed by GC are likely to not be sufficiently soluble in supercritical CO_2 to allow extraction, e.g., many polar pesticides and ionic surfactants are simply not soluble in CO_2 under normal pressures and temperatures. It also becomes less likely that raising the SFE temperature will be useful, since these compounds are often thermally unstable (e.g., many newer pesticides) and/or have such low vapor pressures that no solubility enhancement

occurs. The lack of reports for extracting such analytes makes generalized rules difficult to give. Adding organic modifiers is almost certainly necessary, or alternate fluids could be considered. For example, the ionic surfactant, linear alkylbenzene sulfonates (LAS), cannot not be extracted with pure CO_2, but has been efficiently extracted using high concentrations of methanol modifier (Hawthorne *et al.*, 1991) or using pure $CHClF_2$ (Hawthorne *et al.*, 1992a). In addition, the analyst should consider derivatization procedures as discussed above, especially if analytes must be derivatized in order to perform the analysis.

X.C.2. SFE conditions for biological samples

SFE conditions for biological samples and the extraction of natural product matrices are often determined by the nature of the sample matrix; it's water or lipid content, level of anticipated coextractives, expected quantity of extracted (ppb of toxicant versus 50 wt.% of fat in peanuts), and association of the analyte with non-extractive moieties in the matrix such as carbohydrates or protein matter. For this reason, it is probably fair to say that SFE of biological matter is more complex, requires a diversity of extraction conditions, and is accomplished easier via off-line SFE techniques, than in other areas of application. For example, the simple extraction of fat from a food matrix can be highly dependent on the water content. Extraction of oil from soybeans is accomplished relatively easy by SFE; however, extraction of similar fat moieties from a high water content meat sample takes considerably more sample manipulation than the former case. Likewise, extraction of fat on a 5–20% weight level from certain sample matrices is frequently easier to accomplish than removal of trace lipid species from biological tissues or high fat containing matrices where the bed dynamics may influence the final outcome of the extraction. All of the above cases are manageable, but require that analytical SFE be optimized for the case under consideration, just as it is in liquid extraction. It must also be realized that analytical SFE is an expanding technique with regard to biological and food applications, and every new application may require a somewhat different approach. With this in mind, and the diversity of applications already reported (Dean, 1993), we have selected three generic areas of application to illustrate conditions for conducting SFE on biological samples, namely the determination of a non-volatile specie in a sample matrix (oil in seeds), extraction of trace level volatile species from an accelerated storage seed oil sample, and the determination of a moderately polar analyte (aflatoxins) requiring the use of a cosolvent to affect the extraction from corn. All three of these examples show the applicability of analytical SFE to seed oil matrices and illustrate the diverse possibilities for applying SFE to matrices having very similar properties.

Extraction of oils and fats

This area is chosen as an example of the application of analytical SFE because of its potential widespread utilization, compatibility with SC-CO_2 extraction, its impact on the elimination of using organic solvents in the laboratory environment, and the requirement of an exhaustive SFE of potentially large quantities of extracted analyte. The extraction of such moieties is particularly sensitive to extraction pressure and temperature, as

Table 13 Experimental parameters which influence oil/fat extractions by SFE.

sample size
sample preparation (particle size)
oil content of sample
extraction pressure and temperature
moisture level in sample
coextraction of water
oil dehydration and outgassing
condensation of moisture in collection vessel

indicated by Figure 22; hence, the extraction of a large quantity of lipid matter should be done at an appropriate high pressure and temperature and at an adequately high flow rate to complete the extraction in minimal time (King, 1989a). Approximate weight percents for soybean oil in SC-CO_2 are 0.1 at 193 bar and 80°C, 1.0 at 345 bar and 80°C, and potentially well over 20 at 689 bar and 80°C. Hence, the advantages of conducting the SFE and having the appropriate equipment that can reach higher pressures/temperatures is self evident.

As noted before, high moisture content is inhibitory to completing a quantitative extraction and prohibitive in terms of time. Oil seeds of moderate moisture content can be handled by in-situ desiccation with extraction additives; however, cereal grains and many bakery products are resistant to complete extraction due to complexation of the fat moieties with starch or lipid-carbohydrate complexation. The latter may be partially overcome by the use of specific cosolvents (Knightly, 1989) or extensive hydrolysis of the sample matrix. Newer nutritional labeling methods, based on GC analysis of hydrolyzed and derivatized fatty acids, are compatible with SFE, but the method can be complex and time consuming (House *et al.*, 1994). SFE of lipid matter from biological matrices is often non-selective; hence, minor lipid constituents, such as cholesterol, are difficult to extract individually, since coextracted lipid matter is usually always present in minor amounts. Some SFE speciation can be achieved by employing sorbents in the extraction cell, or post extraction, for isolating the individual lipid species.

Table 13 lists the experimental parameters that are germane to the extraction of oil and fat via SFE. Large sample sizes will require a fluid pumping system that can achieve at least a 4 m/min delivery rate of liquid CO_2. Sample comminution is the rule in extractions of this type; in the case of seed extraction, to break the cell walls that prohibit free access of the extraction fluid to the oil matrix. Coextraction of water may be sufficiently delayed so that no appreciable extraction (as evidenced by turbidity in the oil) will occur in the earlier part of the extraction. However, the extracted fat may contain imbibed fluid still dissolved in the extract or trace levels of water which should be removed via outgassing of the extract, particularly if the determination of fat is being done on a gravimetric basis. An example of such results are shown in Table 14, where the gravimetric-based determination of oil content was determined for three different seed types, including highly moist, wet-milled corn germ and a canola sample containing a very high oil content. Agreement between the SFE and solvent extracted sample is excellent for all three different seed oil types, lending credence to the SFE method (Taylor *et al.*, 1993a).

Table 14 Comparison of extraction of various seed oils SFE vs. Soxhlet.

Oilseed Type	*Weight Percent*	
	SFE	*Soxhlet*
Soybean Flakes	20.6 ± 0.2	20.5 ± 0.2
Canola	39.8 ± 0.5	40.5 ± 0.5
Wet-Milled Corn Germ	48.9 ± 0.5	50.4 ± 1.3

Extraction of volatile matter

The extraction of volatiles and semivolatiles from biological matrices by SFE offers some improvement over other techniques such as headspace or purge/trap methods due to its benign nature. SFE using SC-CO_2 bathes the sample in the extraction cell with a non-oxidative environment and can be accomplished at relatively low temperatures, thereby, avoiding molecular alteration of the analyte during the extraction process. Extractions can frequently be performed under 200 bar and at temperatures slightly above the critical temperature of the extracting fluid, e.g., 35–45°C for SC-CO_2. Extractions conducted at lower pressures also avoids the simultaneous extraction of lipid coextractives which can foul the end analytical method, such as gas chromatography. This makes the technique amenable in both the on-, as well as off-line modes.

Trapping of such volatile and semivolatile species is much more critical than in the case of solutes having low vapor pressures or high melting points. As noted previously, collection devices containing sorbents are frequently favored, but even analyte losses from these beds can occur and must be minimized. Taylor *et al.* (1994) have offered the use of inverse gas chromatographic data as a guide for selecting trapping conditions after SFE in the presence of carbon dioxide. Table 15 shows that the breakthrough volume for a hypothetical case of the passage of 60 liters of expanded CO_2 (approx. 120 ml of L-CO_2) over a Tenax trap for a given extraction time and weight of adsorbent. The data show that at 25°C, several analytes would breakthrough from this sorbent trap and be lost for analysis. Reduction of the trap temperature via sub-ambient cooling should eliminate most of the analyte breakthrough, although quantitative capture of ethanol is still not being accomplished.

Experiments in one of the authors' laboratories has shown that relative to purge/trap methods, SFE produces almost no molecular degradation induced by the extraction and trapping method. For example, desorbing volatiles collected from SC-CO_2 purging of heated canola oil on Tenax by purge/trap methodology produces aldehydes lower than C_6, while such species are absent in the desorption conducted under supercritical fluid conditions with CO_2 (e.g., 158 bar, 50°C). These artifacts can be produced even under supercritical desorption conditions if the temperature is raised to 150°C, indicating the value of the lower extraction temperature coupled with the extraction pressure of SC-CO_2. This type of technique has proven very efficacious in studying the degradation of seed oils via SFE coupled with GC/MS as shown in Figure 27. Here the degradation products in autoxidized oils are clearly evident as a function of time for the exposed canola oil sample, using on-line SFE at 103 bar and 50°C (Snyder, 1995).

Table 15 Utilization of specific retention volume to predict analyte breakthrough off sorbent trap.

Extraction Conditions: CO_2 Flow Rate (25°C, 1 atm) = 2.0 l/min
Weight of Tenax = 2.0 grams
Extraction Time = 30 min
Total Volume of CO_2 (25°C, 1 atm) = 60 l (expanded)

Solute	*Vg* (25°C)	*Vg* (-25°C)
n-Hexane	25.2 (Y)	2862 (N)
n-Octane	344 (N)	
n-Decane	10462 (N)	
n-Dodecane	17916 (N)	
Ethanol	1.48 (Y)	38.8 (Y)
Propanol	8.84 (Y)	448 (N)
N-Butanol	58.2 (Y)	6114 (N)
Dichloromethane	4.06 (Y)	126 (N)
Chlorobenzene	606 (N)	
1,2-Dichloroethane	27.0 (Y)	1616 (N)
1,1,2-Trichloroethane	194 (N)	
Pentanal	164 (N)	
Hexanal	800 (N)	
Nonanal	24580 (N)	
2,4 – Decadienal	574000 (N)	

(Y) = Breakthrough occurred; (N) = Breakthrough does not occur.

Extraction of polar toxicant (analyte) from corn

Extraction of polar analytes from biological matrices by SFE presents some of the same problems as SFE removal of these analytes from environmental matrices. Such analytes may be sparingly soluble in SC-CO_2 alone and be tightly bound to the matrix so as to require the use of a cosolvent along with the primary extraction fluid. However, frequently these analytes occur at trace levels in the sample matrix, so that their solubility in a neat extracting fluid is more than sufficient to permit SFE (Lee *et al.*, 1990). Unfortunately, this is not the case for reasons alluded to in Section III.A. and coupling this fact with the matrix effect makes extraction with SFE difficult, but not impossible. The rationale for pursuing SFE, at least for biological samples, even when using a cosolvent is usually the high extraction recoveries obtained and the overall reduction in solvent relative to an older solvent-intensive method that has been used traditionally.

An illustrative case in point is the extraction of the mycotoxin, aflatoxin B1 from yellow corn which requires the use of a binary modifier to obtain successful recoveries. Extraction with neat SC-CO_2 proved unsuccessful, even at pressures up to 1,034 bar and high temperatures (80°C). Static addition of small aliquots of several modifiers also proved insufficient relative to dynamic addition of the cosolvents. A 2:1 acetonitrile/methanol modifier mixture (Taylor *et al.*, 1993) was then tested using different extraction temperatures, pressures and percent modifier a shown in Table 16. As indicated, 15% of the binary modifier at 345 bar and 80°C proved sufficient to give recoveries equivalent to those

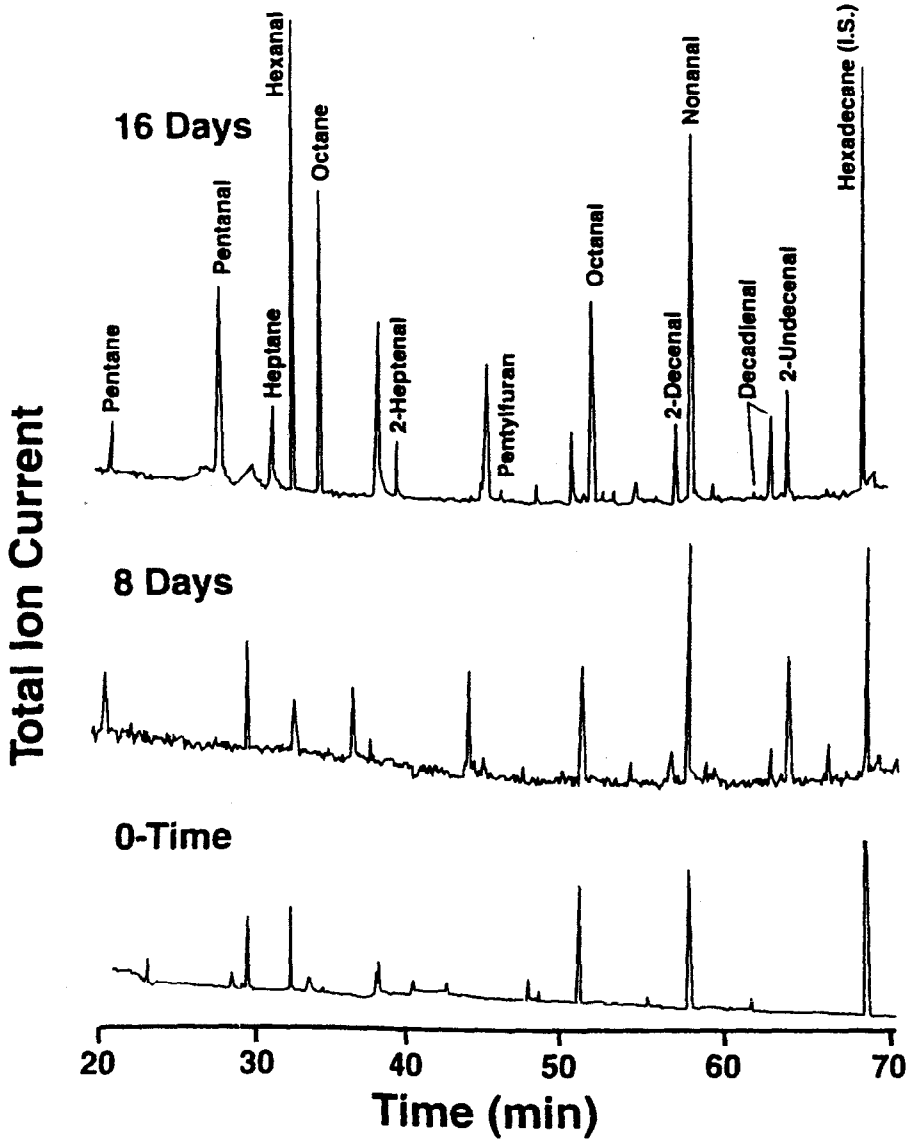

Figure 27 SFE/GC/MS — Total ion current profile chromatograms showing accelerated storage effect at zero, 8 days, and 16 days storage for canola oil.

obtained via solvent extraction. Such scouting for optimal conditions in SFE is not uncommon and can be easily accommodated overnight on automated analytical SFE equipment.

Will these conditions suffice for the same or similar mycotoxins in different matrices and at different levels of contamination? Recent research has shown at much lower levels of aflatoxin in yellow corn (15 ppb versus 600 ppb in the above example), that aflatoxin

Table 16 Screening for optimum conditions — SFE of aflatoxin B_1 from a corn sample.

Pressure (bar)	*Temp. (°C)*	*% Modifier* (vol)*	*Vol Compressed CO_2 (m)*	*Recovery (ppb)*
345	80	5	100	476
345	80	10	100	274
345	80	15	100	595
345	80	20	100	282
517	40	5	100	446
517	40	10	100	502
517	40	15	100	459
517	40	20	100	282
Standard Methods				
CB Method**				449
No Cleanup***				595

* ACN/MeOH (2:1), ** Silica column cleanup of HCCl3 extract, *** No silica column cleanup.

B1 is only recovered at a 60% level using the above optimal conditions. This low recovery may reflect the difficulty in extracting the lower level of trace analyte from the yellow corn matrix. Similar results have also been recorded for the recovery of aflatoxin B1 from white corn, indicating that the method does not have universal applicability to a variety of sample matrices. Using the above approach, extractions were attempted of the more polar aflatoxin B1 metabolite, aflatoxin M1, from beef liver at a 0.3 ppb level! Liver is a notoriously difficult matrix to extract analytes from (see Section III.A.), and the use of cosolvents creates the need for extract cleanup after completion of the extraction. Nevertheless, a reasonably clean extract can be achieved by conducting the extraction at 552 bar and 80°C, using only 3.3 volume percent of 2:1 acetonitrile/methanol modifier, which yields a 86% recovery (Langenfeld *et al.*, 1994). Although such method develop is often arduous, as it is in conventional liquid extraction, it is the above experimental flexibility that makes analytical SFE unique, and such an attractive technique.

SUMMARY

The authors hope that the discussions presented in this chapter will provide readers a firm, practical background in analytical SFE, as well as a basic understanding of the chemistry controlling these extractions. The material presented should help new users avoid many common pitfalls, and hopefully will help readers to understand why the authors are so enthused about this very versatile new approach to extracting analytes from both environmental and biological samples.

REFERENCES

Alexandrou, N. and Pawliszyn, J. (1989) Supercritical fluid extraction for the rapid determination of polychlorinated dibenzo-p-dioxins and dibenzofurans in municipal incinerator fly ash. *Anal. Chem.*, **61**, 2770.

Alexandrou, N., Lawrence, M.J. and Pawliszyn, J. (1992) Cleanup of complex organic mixtures using supercritical fluids and selective adsorbents. *Anal. Chem.*, **64**, 301.

Ashraf-Khorassani, M., Houck, R. and Levy, J.M. (1992) Cryogenically cooled adsorbent trap for off-line supercritical fluid extraction. *J. Chromatogr. Sci.*, **30**, 361.

Ashraf-Khorasanni, M., Taylor, L.T. and Schweighardt, F.K. (1996) Comparison of supercritical fluoroform and carbon dioxide for the extraction of sulfonamides from chicken liver. *J. Assoc. Off. Anal. Chem. Int.*, **79**, in press.

Bartle, K.D., Boddington, T., Clifford, A.A. and Hawthorne, S.B. (1992) The effect of solubility on the kinetics of dynamic supercritical-fluid extraction. *J. Supercrit. Fluids*, **5**, 207.

Bartle, K.D., Clifford, A.A., Hawthorne, S.B., Langenfeld, J.J., Miller, D.J. and Robinson, R. (1990) A model for dynamic extraction using a supercritical fluid. *J. Supercrit. Fluids*, **3**, 143.

Bartle, K.D., Clifford, A.A., Jafar, S.A. and Shilstone, G.F. (1991) Solubilities of solids and liquids of low volatility in supercritical carbon dioxide. *J. Phys. Chem. Ref. Data*, **20**, 713.

Bøwadt, S., unpublished results.

Bøwadt, S. and Hawthorne, S.B. (1995) SFE in environmental analysis. *J. Chromatogr. A*, **703**, 549.

Bøwadt, S. and Johansson, B. (1994) Analysis of PCBs in sulfur-containing sediments by off-line supercritical fluid extraction and HRGC-ECD. *Anal. Chem.*, **66**, 667.

Bøwadt, S., Johansson, B., Pelusio, F., Larsen, B.R. and Rovida, C. (1994) Solid-phase trapping of polychlorinated biphenyls in supercritical fluid extraction. *J. Chromatogr.*, **662**, 424.

Bøwadt, S., Pelusio, F., Montanarella, L., Larsen, B. and Kapila, S. (1993) Trapping techniques in supercritical fluid extraction. *J. Trace and Microprobe Techniques*, **11**, 117.

Burford, M.D., Hawthorne, S.B. and Miller, D.J. (1993) Evaluation of drying agents for off-line supercritical fluid extraction. *J. Chromatogr. A*, **657**, 413.

Burford, M.D., Hawthorne, S.B. and Miller, D.J. (1993) Extraction rates of spiked versus native PAHs from heterogeneous environmental samples using supercritical fluid extraction and sonication in methylene chloride. *Anal. Chem.*, **65**, 1497.

Burford, M.D., Hawthorne, S.B. and Miller, D.J. (1994) Analysis of volatile organics by supercritical fluid extraction coupled to gas chromatography. I. Optimization of chromatographic parameters. *J. Chromatogr. A*, **685**, 79.

Burford, M.D., Hawthorne, S.B., Miller, D.J. and Braggins, T. (1992) Comparison of methods to prevent restrictor plugging during off-line supercritical extraction. *J. Chromatogr.*, **609**, 321.

Camel, V., Tambuté and Caude, M. (1993) Analytical-scale supercritical fluid extraction: a promising technique for the determination of pollutants in environmental matrices. *J. Chromatogr.*, **642**, 263.

Carpenter, D.E., Ngeh-Ngwainbi, J. and Lee, S. (1993) Lipid Analysis. In *Methods for Nutritional Labeling*, edited by D.M. Sullivan and D.E. Carpenter, Chapter 5, pp. 85–104. AOAC International, Arlington, VA.

Chester, T.L. and Innis, D.P. (1986) Separation of oligo- and polysaccharides by capillary supercritical fluid chromatography. *J. High Resolut. Chromatogr. & Chromatogr. Commun.*, **9**, 209–212.

Chester, T.L., Pinkston, J.D. and Raynie, D.E. (1994) Supercritical fluid chromatography and extraction. *Anal. Chem.*, **66**, 106R.

Clifford, A.A., Burford, M.D., Hawthorne, S.B., Langenfeld, J.J. and Miller, D.J. (1995) The effect of the matrix on the kinetics of dynamic supercritical fluid extraction. *J. Chem. Soc. Faraday Trans.*, **91**, 1333.

David, F., Verschuere, M. and Sandra, P. (1992) Off-line supercritical fluid extraction-capillary GC applications in environmental analysis. *Fresenius' J. Anal. Chem.*, **344**, 479.

Dean, J.R. (1993) Applications of supercritical fluids in food science. In *Applications of Supercritical Fluids in Industrial Analysis*, edited by J.R. Dean, Chapter 6, pp. 130–158. Blackie Academic & Professional, London.

Deye, J.F., Berger, T.A. and Anderson, A.G. (1990) Nile red as a solvatochromic dye for measuring solvent strength in normal liquids and mixtures of normal liquids with supercritical and near critical fluids. *Anal. Chem.*, **62**, 615.

Field, J.A., Miller, D.J., Field, T.M., Hawthorne, S.B. and Giger, W. (1992) Quantitative determination of sulfonated aliphatic and aromatic surfactants in sewage sludge by ion-pair/supercritical fluid extraction and derivatization gas chromatography/mass spectrometry. *Anal. Chem.*, **64**, 3161–3167.

(1993) *Fed. Regist.*, **58**, 631–2964.

France, J.E, King, J.W. and Snyder, J.M. (1991) Supercritical fluid-based cleanup technique for the separation of organochlorine pesticides from fats. *J. Agric. Food Chem.*, **39**, 1871–1874.

France, J.E. and King, J.W. (1991) Supercritical fluid extraction/enzyme assay. A novel technique to screen for pesticide residues in meat products. *J. Assoc. Off. Anal. Chem.*, **74**, 1013–1016.

Giddings, J.C., Myers, M.N. and King, J.W. (1969) Dense gas chromatography at pressures to 2000 atmospheres. *J. Chromatogr. Sci.* **7**, 276.

Goodrum, J.W. and Kilgo, M.B. (1989) Rapeseed oil recovery by CO_2 solvent: Recovery kinetics and extraction modeling. *Am. Soc. Agric. Engrs.*, **32**, 727–731.

Hawthorne, S.B. (1992). In *Analysis with Supercritical Fluids: Extraction and Chromatography*, edited by B. Wenclawiak, pp. 67. Springer, Berlin.

Hawthorne, S.B. and Miller, D.J. (1994) Direct comparison of Soxhlet and low- and high-temperature supercritical CO_2 extraction efficiencies of organics from environmental solids. *Anal. Chem.*, **66**. 4005.

Hawthorne, S.B., Galy, A.B., Schmitt, V.O. and Miller, D.J. (1995) Effect of SFE flow rate on extraction rates: an approach to classifying sample extraction behavior. *Anal. Chem.*, **67**, 2723.

Hawthorne, S.B., Langenfeld, J.J., Miller, D.J. and Burford, M.D. (1992) Comparison of supercritical $CHClF_2$, N_2O and CO_2 for the extraction of polychlorinated biphenyls and polycyclic aromatic hydrocarbons. *Anal. Chem.*, **64**, 1614.

Hawthorne, S.B., Miller, D.J., Burford, M.D., Langenfeld, J.J., Eckert-Tilotta, S.E. and Louie, P.K. (1993) Factors controlling quantitative supercritical fluid extraction of environmental samples. *J. Chromatogr.*, **642**, 301.

Hawthorne, S.B., Miller, D.J., Walker, D.D., Whittington, D.E. and Moore, B.L. (1991) Quantitative extraction of linear alkylbenzenesulfonates using supercritical carbon dioxide and a simple device for adding modifiers. *J. Chromatogr.*, **541**, 185.

Hawthorne, S.B., Riekkola, M.-L., Serenius, K., Holm, Y., Hiltunen, R. and Hartonen, K. (1993) Comparison of hydrodistillation and supercritical fluid extraction for the determination of essential oils in aromatic plants. *J. Chromatogr.*, **634**, 297.

Heglund, D.L., Tilotta, D.C., Hawthorne, S.B. and Miller, D.J. (1994) Simple fiber-optic interface for on-line supercritical fluid extraction-fourier transform infrared spectrometry. *Anal. Chem.*, **66**, 3543.

Hopper, M.L. and King, J.W. (1991) Enhanced supercritical fluid carbon dioxide extraction of pesticides from foods using pelletized diatomaceous earth. *J. Assoc. Off. Anal. Chem.*, **74**, 661–666.

Hopper, M.L. and King, J.W. (1992) Supercritical fluid extraction enhancer, U.S. Patent 5,151,188.

Hopper, M.L., King, J.W., Johnson, J.H., Serino, A.A. and Butler, R.J. (1995) Supercritical fluid extraction (SFE): Multivessel extraction of food items in the FDA total diet study (TDS). *J. Assoc. Off. Anal. Chem. Int.*, **78**, 1072–1079.

Horwitz, W., Kamps, L.R. and Boyer, K.W. (1980) Quality assurance in the analysis of foods for trace constituents. *J. Assoc. Off. Anal. Chem.*, **63**, 1344–1354.

House, S.D., Larson, P.A., Johnson, R.R., DeVries, J.W. and Martin, D.L. (1994) Gas chromatography determination of total fat extracted from food samples using hydrolysis in the presence of an antioxidant. *J. Assoc. Off. Anal. Chem.*, **77**, 960–965.

Howard, A.L. and Taylor, L.T. (1993) Considerations for analytical supercritical fluid extraction of sulfonyl ureas employing a modified fluid. *J. High Resolut. Chromatogr.*, **16**, 39.

Janda, V., Bartle, K.D. and Clifford, A.A. (1993) Supercritical fluid extraction in environmental analysis. *J. Chromatogr.*, **642**, 283.

Johnston, K.P. (1989) New directions in supercritical fluid science and technology. In *Supercritical Fluid Science and Technology*, edited by K.P. Johnston and J.M.L. Penninger, Chapter 1, pp. 1–12. American Chemical Society, Washington, DC.

King, J.W. (1989) Extraction of fat tissue from meat products with supercritical carbon dioxide. *J. Agric. Food Chem.*, **37**, 951–954.

King, J.W. (1989) Fundamentals and applications of supercritical fluid extraction in chromatographic science. *J. Chromatogr. Sci.*, **27**, 355–364.

King, J.W. (1993) Analysis of fats and oils by SFE and SFC. *INFORM*, **4**, 1089–1098.

King, J.W. (1995) Analytical-process supercritical fluid extraction: a synergestic combination for solving analytical and laboratory scale problems. *Trends Anal. Chem.*, **14**, 474–481.

King, J.W. and Hopper, M.L. (1992) Analytical supercritical fluid extraction: current trends and future Vistas. *J. Assoc. Off. Anal. Chem.*, **75**, 375.

King, J.W., Eller, F.J., Snyder, J.M., Johnson, J.H., McKeith, F.K. and Stites, C.R. (1996) Extraction of fat from ground beef for nutrient analysis using analytical supercritical fluid extraction, *J. Agric. Food Chem.*, **44**, in press.

King, J.W., Hopper, M.L., Luchtefeld, R.G., Taylor, S.L. and Orton, W.L. (1993) Optimization of experimental conditions for the supercritical carbon dioxide extraction of pesticide residues from grains. *J. Assoc. Off. Anal. Chem. Int.*, **76**, 857–864.

King, J.W., Johnson, J.H. and Eller, F.J. (1995) Effect of supercritical carbon dioxide pressurized with helium on solute solubility during supercritical fluid extraction. *Anal. Chem.*, **67**, 2288–2291.

Knightly, W.H. (1989) Lecithin in baking applications. In *Lecithins: Sources, Manufacture, and Uses*, edited by B.F. Szuhaj, Chapter 11, pp. 174–178. American Oil Chemists' Society, Champaign, IL.

Laintz, K.E., Wai, C.M., Yonker, C.R. and Smith, R.D. (1991) Solubility of fluorinated metal diethyldithiocarbamates in supercritical carbon dioxide. *J. Supercrit. Fluids*, **4**, 194–198.

Langenfeld, J.J., Burford, M.D., Hawthorne, S.B. and Miller, D.J. (1992) Effects of collection solvent parameters and extraction cell geometry on supercritical fluid extraction efficiencies. *J. Chromatogr.*, **594**, 297.

Langenfeld, J.J., Hawthorne, S.B., Miller, D.J. and Burford, M.D. (1992) Comparison of supercritical $CHClF_2$, N_2O, and CO_2 for the extraction of polychlorinated biphenyls and polycyclic aromatic hydrocarbons. *Anal. Chem.*, **64**, 1614.

Langenfeld, J.J., Hawthorne, S.B., Miller, D.J. and Pawliszyn, J. (1993) Effects of temperature and pressure on supercritical fluid extraction efficiencies of polycyclic aromatic hydrocarbons and polychlorinated biphenyls. *Anal. Chem.*, **65**, 338.

Langenfeld, J.J., Hawthorne, S.B., Miller, D.J. and Pawliszyn, J. (1994) Role of modifiers for analytical-scale supercritical fluid extraction of environmental samples. *Anal. Chem.*, **66**, 909.

Langenfeld, J.J., Hawthorne, S.B., Miller, D.J. and Pawliszyn, J. (1995) Kinetic study of supercritical fluid extraction of organic contaminants from heterogeneous environmental samples with carbon dioxide and elevated temperatures. *Anal. Chem.*, **34**, 1727.

Lee, A.K.K., Bulley, N.R., Fattori, M. and Meisen, A. (1986) Modelling of supercritical carbon dioxide extraction of canola oilseed in fixed beds. *J. Am. Oil Chem. Soc.*, **63**, 921–925.

Lee, M.L. and Markides, K.E. (eds.) (1990) *Analytical Supercritical Fluid Chromatography and Extraction*, Chapter 5, p. 321–322. Chromatography Conferences, Inc., Provo, UT.

Levy, J.M. and Ashraf-Khorassani (1992). In *Hyphenated Techniques in Supercritical Fluid Chromatography and Extraction (J. Chromatogr. Library)*, edited by K. Jinno, Vol. 53, pp. 197.

Levy, J.M. and Houck, R.K. (1993). New developments in off-line collection for SFE. *Amer. Lab.*, **25**(4), 36R–36Y.

Levy, J.M., Cavalier, R.A., Bosch, T.N., Rynaski, A.F. and Huhak, W.E. (1989) Multi-dimensional supercritical fluid chromatography and supercritical fluid extraction. *J. Chromatogr. Sci.*, **27**, 341–346.

Lin, Y. and Wai, C.M. (1994) Supercritical fluid extraction of lanthanides with fluorinated beta-diketones and tributyl phosphate. *Anal. Chem.*, **66**, 1971–1975.

Lopez-Avila, V., Benedicto, J., Dodhiwla, N.S., Young, R. and Beckert, W.F. (1992) Development of an off-line SFE-IR method for petroleum hydrocarbons in soils. *J. Chromatogr. Sci.*, **30**, 335–343.

Lopez-Avila, V., Dodhiwala, N.S. and Beckert, W.F. (1990) Supercritical fluid extraction and its application to environmental analysis. *J. Chromatogr. Sci.*, **28**, 468.

Lopez-Avila, V., Dodhiwala, N.S. and Beckert, W.F. (1993) Developments in the supercritical fluid extraction of chlorophenoxy acid herbicides from soil samples. *J. Agric. Food Chem.*, **41**, 2038–2044.

Louie, P.K.K., Timpe, R.C., Hawthorne, S.B. and Miller, D.J. (1993) Determination of elemental sulphur in coal by supercritical fluid extraction and gas chromatography with atomic emission detection. *Fuel*, **72**, 225.

Maeda, T. and Hobo, T. (1992) Jinno, K. (ed.), *Hyphenated Techniques in Supercritical Fluid Chromatography and Extraction* (J. Chromatogr. Library, Vol. 53), pp. 255.

Maxwell, R.J., Lightfield, A.R. and Stolker, A.A.M. (1995) An SPE column-teflon sleeve assembly for in-line retention during supercritical fluid extraction of analytes from biological matrices. *J. High Resolut. Chromatogr.*, **18**, 231–234.

McHugh, M.A. and Krukonis, V.J. (1994) *Supercritical Fluid Extraction* – 2nd Ed., Butterworth-Heinemann, Boston, MA, pp. 367–370.

McNally, M.E.P. (1995) Advances in environmental SFE. *Anal. Chem.*, **67**, 308A–315A.

Meyer, A. and Kleiböhmer, W. (1993) Supercritical fluid extraction of polycyclic aromatic hydrocarbons from a marine sediment and analyte collection via liquid-solid trapping. *J. Chromatogr. A*, **657**, 327.

Miller Schantz, M. and Chesler, S.N. (1986) Supercritical fluid extraction procedure for the removal of trace organic species from solid samples. *J. Chromatogr.*, **363**, 397.

Miller, D.J. and Hawthorne, S.B. (1995) Determination of solubilities of organic solutes in supercritical CO_2 by on-line flame ionization detection. *Anal. Chem.*, **67**, 273.

Miller, D.J., Hawthorne, S.B. and Clifford, A.A. (1997) Solubility of chlorinated hydrocarbons in supercritical carbon dioxide from 313 K to 413 K and pressures from 150 to 450 bar. *J. Supercrit. Fluids*, **10**, 57–63.

Miller, D.J. and Hawthorne, S.B. (1996) Solubility of polycyclic aromatic hydrocarbons in supercritical carbon dioxide from 313 K to 523 K and pressures from 100 bar to 450 bar. *J. Chem. Eng. Data*, **41**, 779–786.

Mulcahey, L.J. and Taylor, L.T. (1992) Collection efficiency of solid surface and sorbent traps in supercritical fluid extraction with modified carbon dioxide. *Anal. Chem.*, **64**, 2352.

Mulcahey, L.J., Hedrick, J.L. and Taylor, L.T. (1991) Collection efficiency of various solid-phase traps for off-line supercritical fluid extraction. *Anal. Chem.*, **63**, 2225.

Onuska, F.I. and Terry, K.A. (1991) Supercritical fluid extraction of polychlorinated diobenzo-p-dioxins from municipal incinerator fly ash. *J. High Resolut. Chromatogr.*, **14**, 829.

Pawliszyn, J. (1993) Kinetic model of supercritical fluid extraction. *J. Chromatogr. Sci.*, **31**, 31.

Peker, H., Srinivasan, M.P., Smith, J.M. and McCoy, B.J. (1992) Caffeine extraction rates from coffee beans with supercritical carbon dioxide. *AIChE J.*, **38**, 761–770.

Raynie, D.E. (1993) Warning concerning the use of nitrous oxide in supercritical fluid extraction. *Anal. Chem.*, **65**, 3127–3128.

Reindl, S. and Höfler, F. (1994) Optimization of the parameters in supercritical fluid extraction of polynuclear aromatic hydrocarbons from soil samples. *Anal. Chem.*, **66**, 1808.

Robertson, A.M. and Lester, J.N. (1994) Supercritical fluid extraction of striazines and phenylurea herbicides from sediment. *Environ. Sci. Technol.*, **28**, 346.

Selim, M.I. and Tsuei. (1993) Development and optimization of a supercritical fluid extraction method for the analysis of aflatoxin B1 in grain dust. *Am. Ind. Hyg. Assoc. J.*, **54**, 135–141.

Snyder, J.M. (1995) Volatile analysis of oxidized oils by a direct supercritical fluid extraction method. *J. Food Lipids*, **2**, 25–33.

Snyder, J.M. and King, J.W. (1994) Analysis of volatile compounds from supercritical extracted soybeans by headspace gas chromatography and thermal desorption of a polymer adsorbent. *J. Sci. Food Agric.*, **64**, 257–263.

Snyder, J.M., King, J.W. and Jackson, M.A. (1996) Analytical supercritical fluid extraction using lipase catalysis: Conversion of different lipids to methyl esters and effect of moisture. *J. Am. Oil Chem. Soc.*, submitted for publication.

Snyder, J.M., King, J.W. and Jackson, M.A. (1996) Fat content for nutritional labeling by supercritical fluid extraction and an on-line lipase catalyzed reaction. (1996) *J. Chromatogr.*, in press.

Snyder, J.M., King, M.W., Rowe, L.D. and Woerner, J.A. (1993) Supercritical fluid extraction of poultry tissues containing incurred pesticide residues. *J. Assoc. Off. Anal. Chem. Int.*, **76**(4), 888–892.

Snyder, L.R. (1971) The role of the mobile phase in liquid chromatography. In *Modern Practice of Liquid Chromatography*, edited by J.J. Kirkland, Chapter 4, pp. 135–149. Wiley-Interscience, New York.

Stahl, E. and Willing, E. (1980) Extraktion labiler naturstoffe mit uberkritischen gasen. *Pharm. Ind.*, **42**, 1136–1139.

Stahl, E., Quirin, K.-W. and Gerard, D. (1988) *Dense gases for extraction and refining*, p. 177. Springer-Verlag, Berlin.

Taylor, S.L. and King, J.W. (1994) Supercritical fluid extraction of organochlorine pesticides using trifluoromethane. *Air Products Application Bulletin #320–9431.*

Taylor, S.L., King, J.W. and Abel, S.E. (1994) Using inverse gas chromatographic measurements for the optimization of collection conditions in analytical SFE. Abstracts of the *5th Int'l. Symp. SFC and SFE*, Baltimore, MD, January, 1994, D–16.

Taylor, S.L., King, J.W. and List, G.R. (1993) Determination of oil content in oilseeds by analytical supercritical fluid extraction. *J. Am Oil Chem. Soc.*, **70**, 437–439.

Taylor, S.L., King, J.W. and Snyder, J.M. (1994) Tandem supercritical extraction/chromatographic studies of the desert botanical species, Dalea spinosa. *J. Microcol. Sep.*, **6**, 467–473.

Taylor, S.L., King, J.W., Greer, J.I. and Richard, J.L. (1996) Supercritical fluid extraction of aflatoxin M_1 from beef liver, *J. Food Protect*, submitted for publication.

Taylor, S.L., King, J.W., Richard, J.L. and Greer, J.I. (1993) Analytical-scale supercritical fluid extraction of aflatoxin B1 from field-inoculated corn. *J. Agric. Food Chem.*, **41**, 910–913.

Tsirule, K.I. and Tyunina, E.L. (1994) Viscoelastic and deformational properties of polymers under high pressures. In *High Pressure Chemistry and Physics of Polymers*, edited by A.L. Kovarskii, p. 77. CRC Press, Boca Raton, FL.

U.S. Environmental Protection Agency Method 8270A, *SW-846 Methods Manual*, 3rd ed. 1992.

VanHorne, K.C. (Ed.). (1985) *Sorbent Extraction Technology Handbook*, Analytichem International, Inc., Harbor City, CA.

Vannoort, R.W., Chervet, J.P., Lingeman, H., de Jong, G.J. and Brinkman, U.A.Th. (1990) Coupling of supercritical fluid extraction with chromatographic techniques. *J. Chromatogr.*, **505**, 45.

White, D.C., Nivens, D.E., Ringelberg, D., Hedrick, D. and Hawthorne, S.B. (1991) SFE/derivatization for rapid GC/MS identification of bacteria and bacterial products. Abstracts of the *3rd Int'l Symp. SFC and SFE*, Park City, UT, p. 43–44.

Yang, Y., Bøwadt, S., Hawthorne, S.B. and Miller, D.J. (1995) Subcritical water extraction of polychlorinated biphenyls from soil and sediment. *Anal. Chem.*, **67**, 4571.

Yang, Y., Gharaibeh, A., Hawthorne, S.B. and Miller, D.J. (1995) Combined temperature/modifier effects on supercritical CO_2 extraction efficiencies of polycyclic aromatic hydrocarbons from environmental samples. *Anal. Chem.*, **67**, 641.

Yang, Y., Hawthorne, S.B. and Miller, D.J. (1995) Comparison of sorbent and solvent trapping after supercritical fluid extraction of volatile petroleum hydrocarbons from soil. *J. Chromatogr. A*, **699**, 265.